

FIG. 1

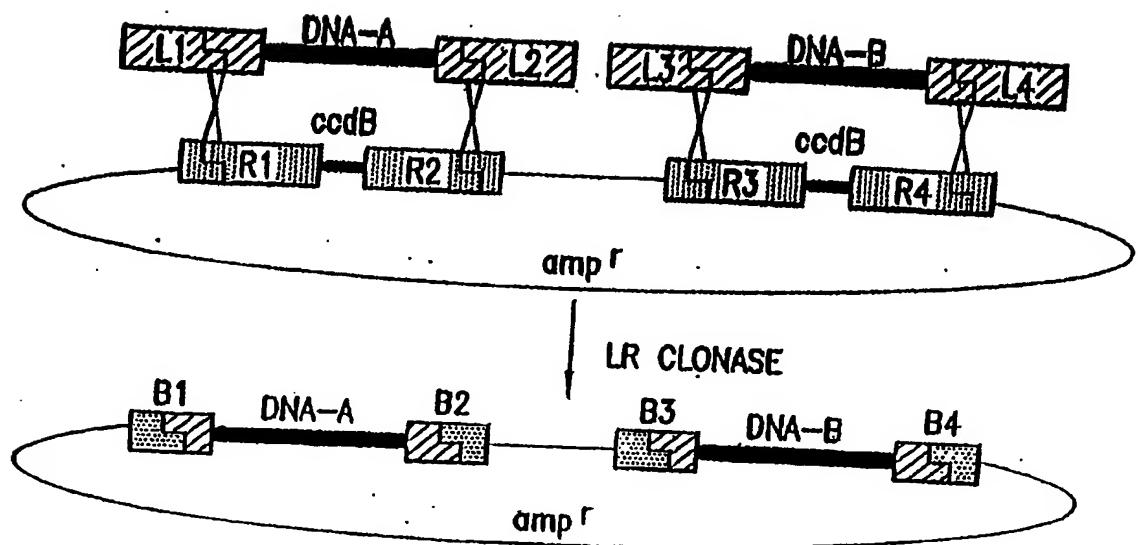


FIG. 2

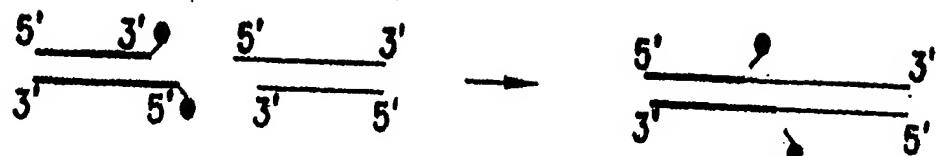


FIG. 3A



FIG. 3B

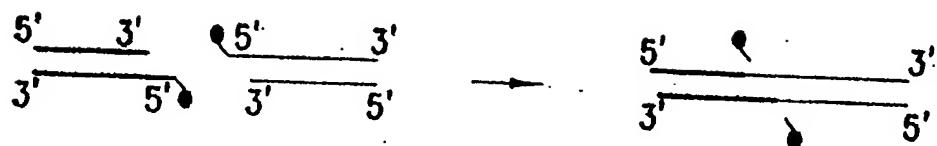


FIG. 3C

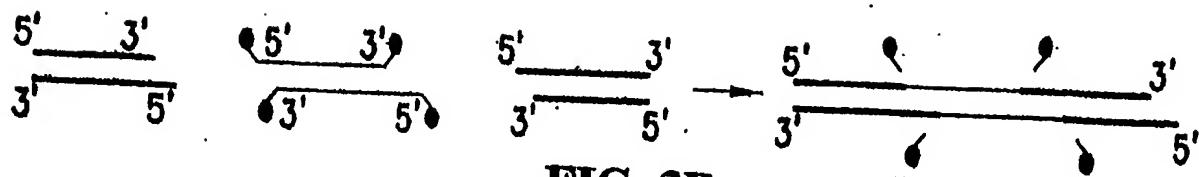
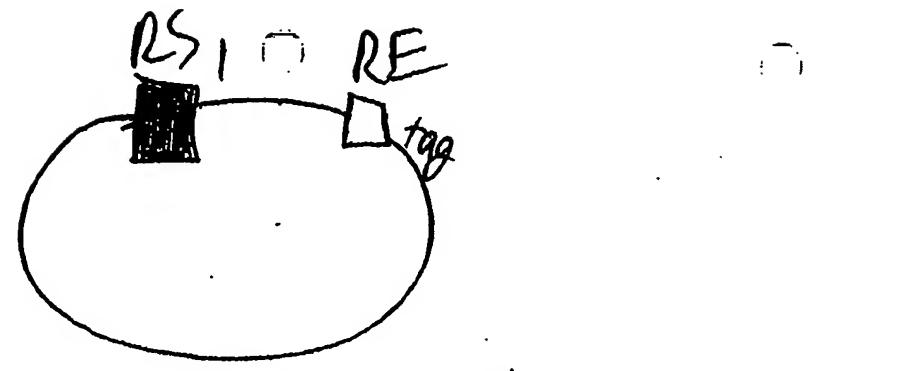
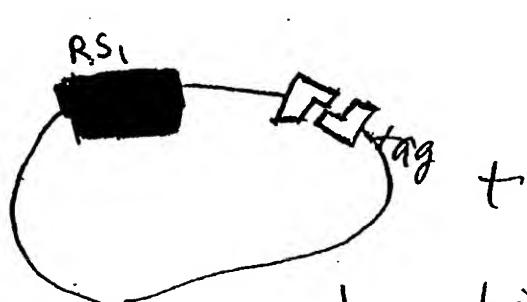


FIG. 3D



↓ RE Digest



+ Ligase + Recombination proteins

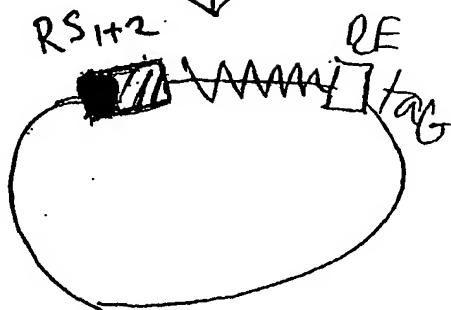


Fig. 4

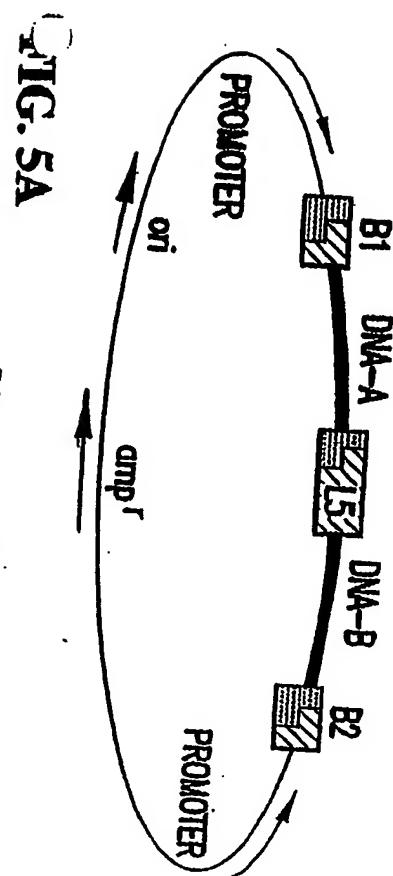


FIG. 5A

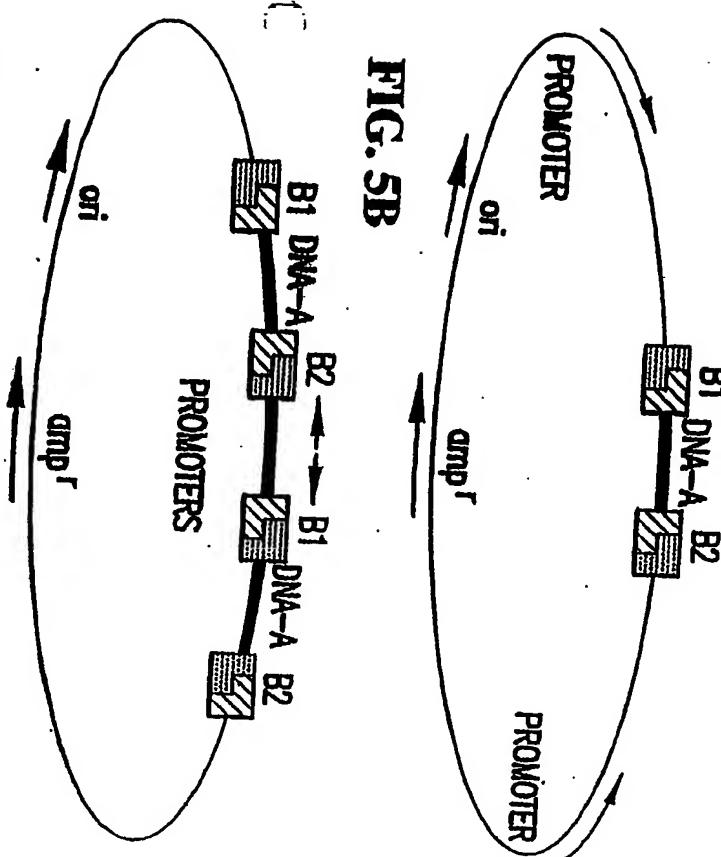


FIG. 5B

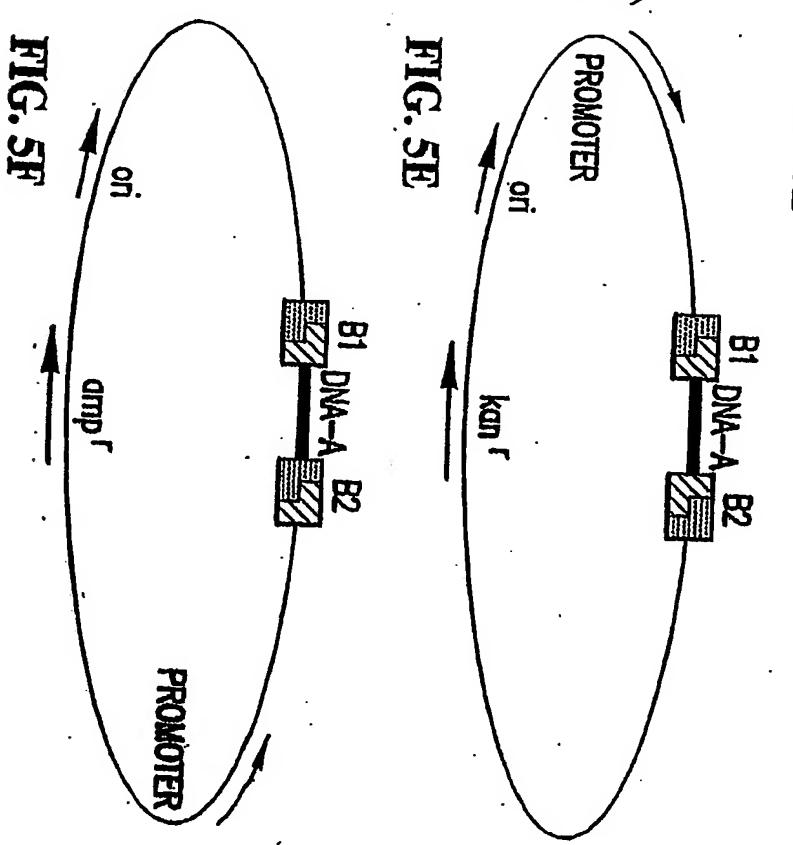


FIG. 5D

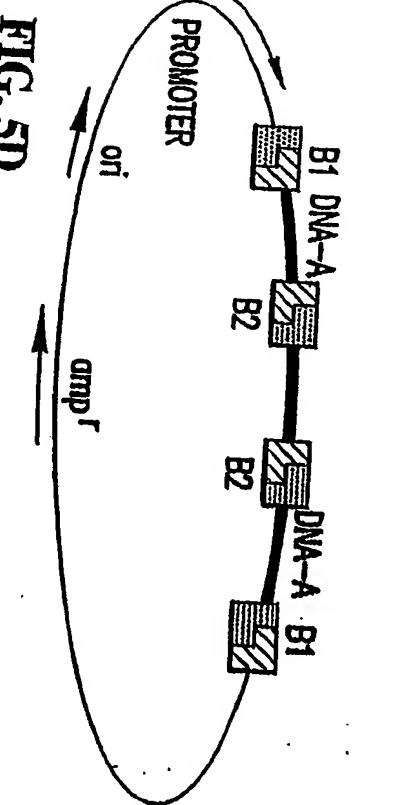


FIG. 5C

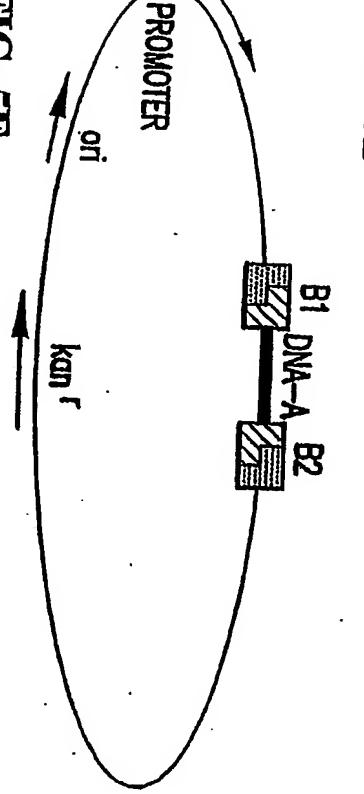


FIG. 5E

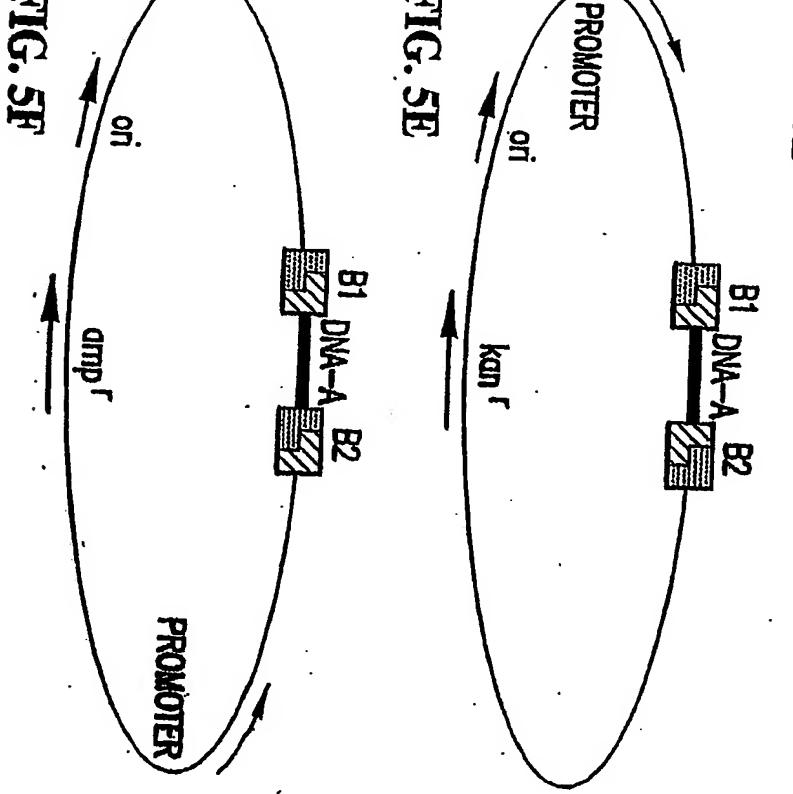


FIG. 5F

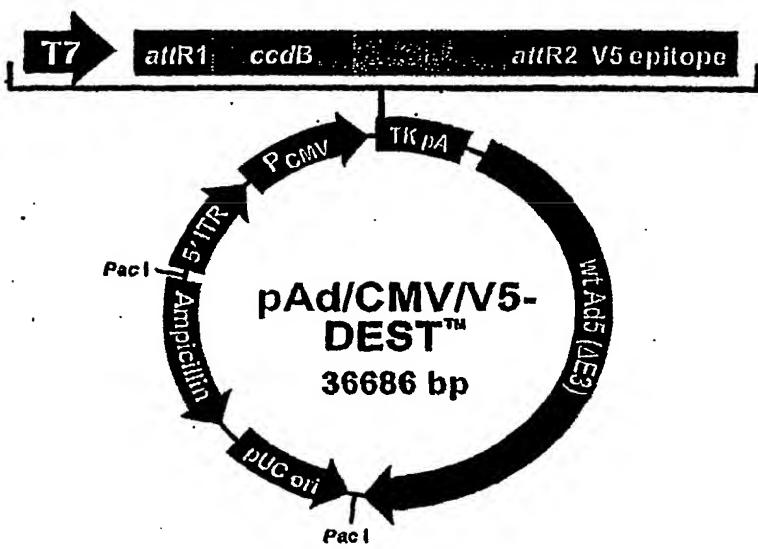


FIG. 6

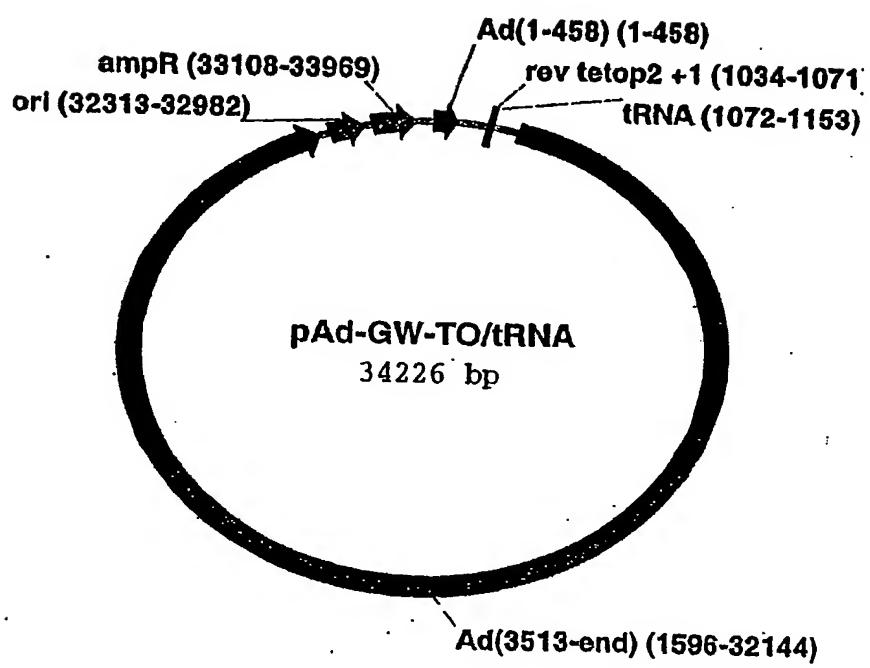


FIG. 7

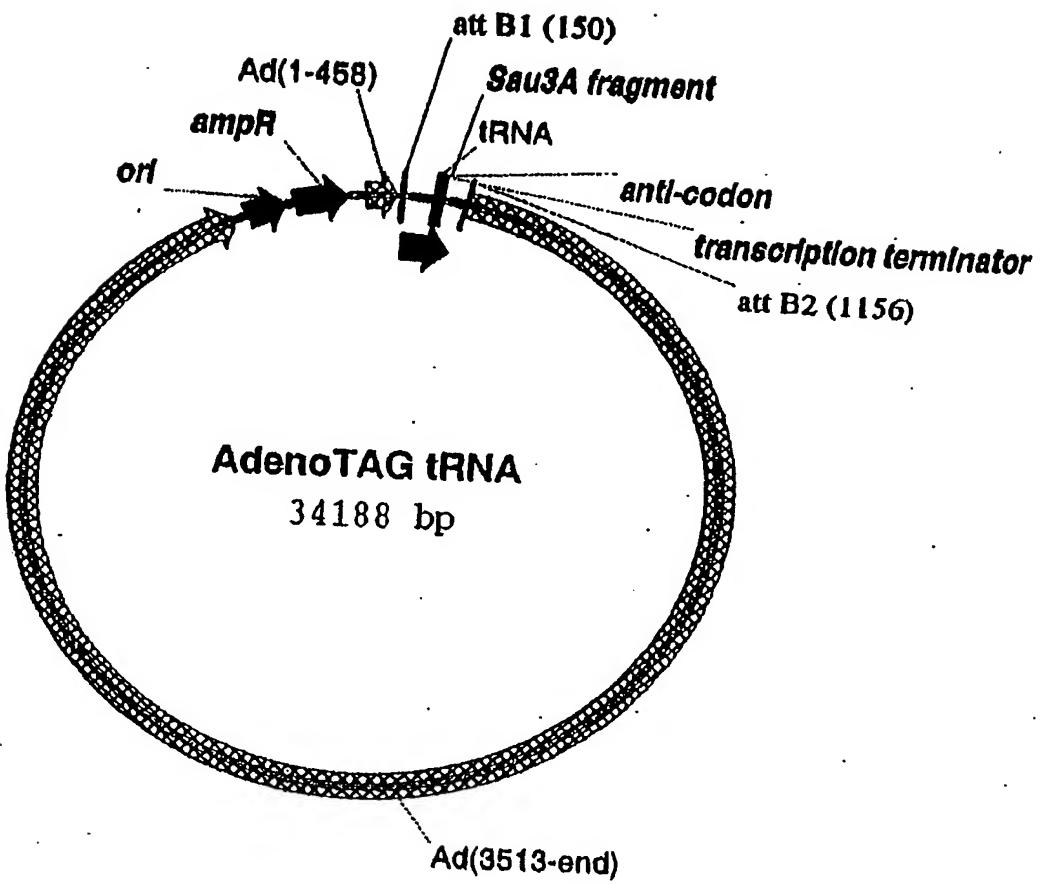


FIG. 8

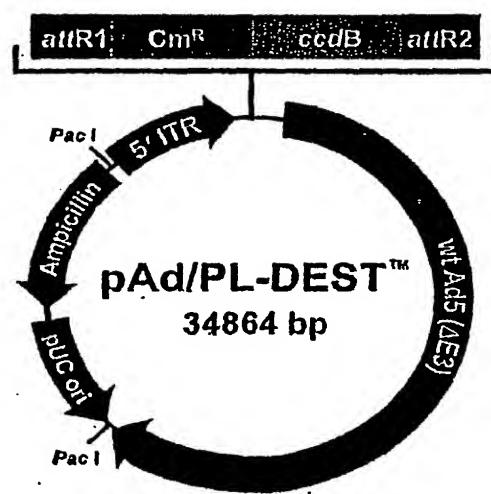


FIG. 9

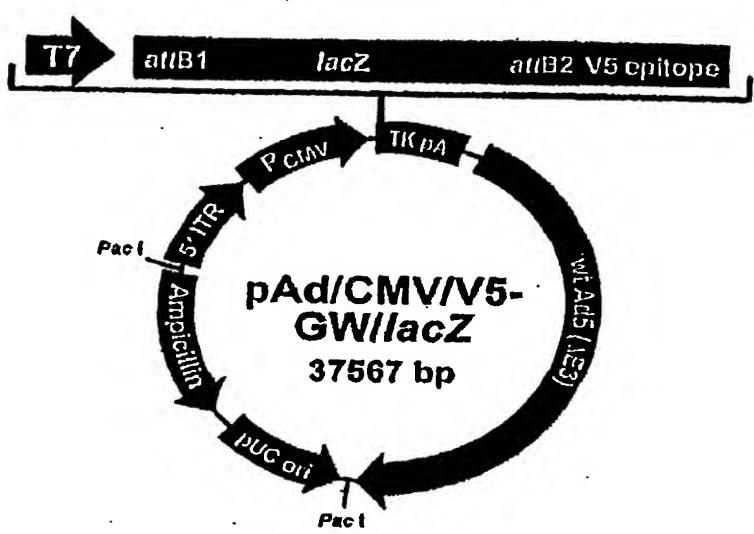


FIG. 10

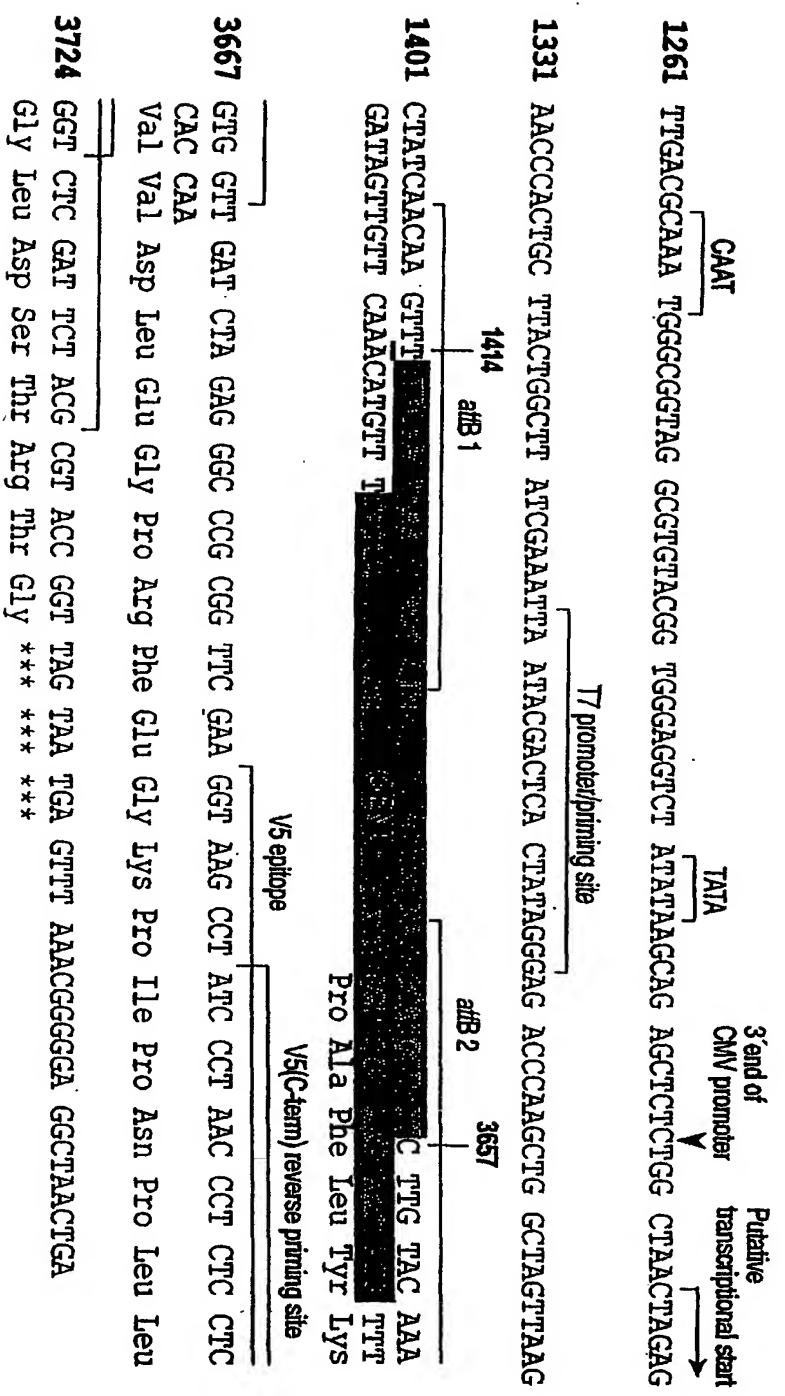


FIG. 11

pad forward priming site

341 TATTTGCTTA GGGCCCCGGG GACTTGACC GTTACGTGG AGACTCGCCC AGGTGTTTT CTCAGGTGT

3' end of Ad5 L-TR and ψ sequences

411 TTCCGGGTTC CGGGTCAAAAG TTGGCGTTTT ATTATTATAAG TCAGTCGAAG CTGGATCCG GTACCTCTAG

519
a β B1

481 ATTTCTCGAG CGGCCGCTAG CGACATGGAT CACAAGTTT
2202 a β B2
GTGTCAAC ATGTTT

pad reverse priming site

CTTGTACAA ACTGGTGATC GATTGACAG ATCACTGAAA TGTGGGGCG TGGCTTAAGG

CTT TCACCAACTA

2261 GTGGGAAGA ATATATAAGG

FIG. 12

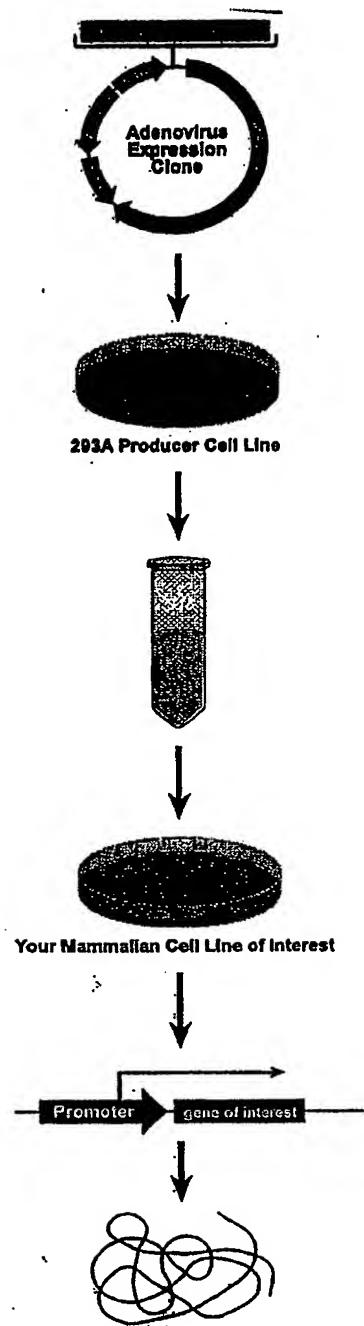


FIG. 13

□

□

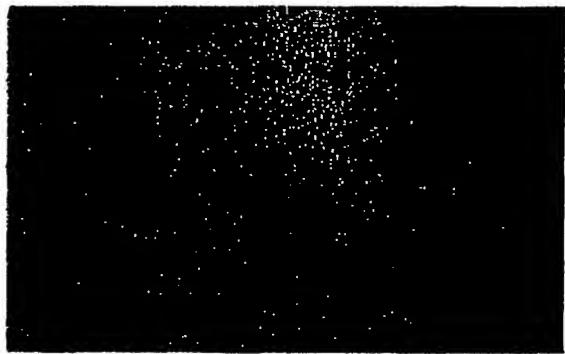


FIG. 14A



FIG. 14B

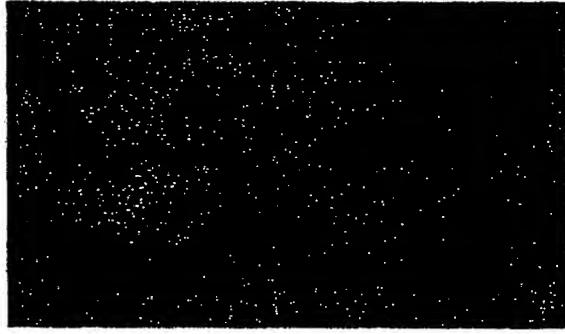


FIG. 14C

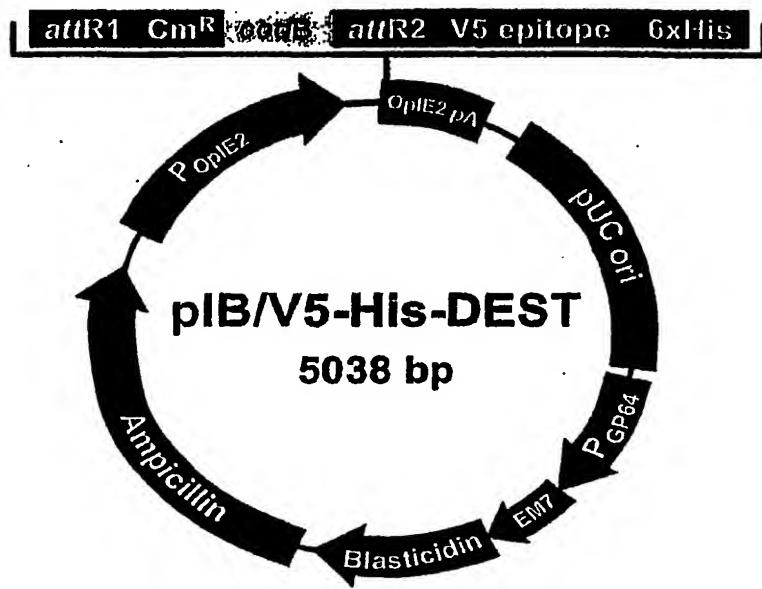


FIG. 15

1 GGATCATGAT GATAAACAAAT GTATGGTGCT AATGGTTGCTT CAACAAACAAT TCTGTGTTGAAC
 61 TGTGTGTTCA TGTGTTGCCAA CAAGCACCTT TATACTCGGT GGCTCCCA CCACCAACTT
 121 TTTTGCACAG CAAAAAAACA CGCTTTGCA CGGGGGCCA TACATAGTAC AACTCTACG
 181 TTTCGTAGAC TATTTCACAT AAATAAGTCTA CACCGTTGTA TACGCTCCAA ATACACTACC
 241 ACACATTGAA CCTTTTGTCA GTGCAAAAAA GTACGTGTGC GCAGTCACGT AGGCCGGCCT
 301 TATCGGGTGC CGTCCCTGTC CGTAGGAATC ACATTTATCGG ACCGGAGGAG TGTGTCTTA
 361 TCGTGTACAGG AGGCCAGCTT CCTGTGTGTC TAACCGCAGC CGGACGCCAAC TCCCTTATCGG
 421 AACAGGACGC GCCTCCATAT CAGCCGCCTG TTATCTCATG CGCGTGACCG GACACGGGC
 481 GCCCGTCCCCG CTTATCGCGC CTATAAATAC AGCCGGAAAC GATCTGGTAA ACACAGGTGA
 541 ACAGGATATCTG TTCGAATTAA

Start of Transcription
 TATA

FIG. 16

Start of transcription ←

487 CTTATCGGCC **TATA Box** OpiE2 Forward priming site

547 TTCGAATTAA AAGCTTGATA TCGAATTCCCT GCAAGCCCAGC GCTGGATCCT CGATCACAAAG
AAGCTTAAAT TTGAACTAT AGCTTAAGGA CGTCTGGTCG CGACCTAGGA GCTAGTGTTC

607 TTI **attB1** AACATGTTT

609

2292 Pro Ala Phe Leu Tyr Lys Val Val
... C TIG TAC AAA GTG GTG
... TTT CAC CAC

attB2

V5 epitope

2308 Ile Asp Pro GLY Leu Glu Pro Arg Phe Glu GLY Lys Pro Ile Pro Asn
ATC GAC CCG GGT CTA GAG GGC CCG CGG TTC GAA GGT AAG CCT ATC CCT AAC
TAG CTG GGC CCA GAT CTC CCG GCC AAG CTT CCA TTC GGA TAG GGA TTG

2359 Pro Leu Gly Leu Asp Ser Thr Arg Thr GLY His His His His His
CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT CAT CAT CAC CAC CAT
GGA GAG GAG CCA GAG CTA AGA TGC GCA TGG CCA GTA GTA GTG GTA GTG GTA

OpiE2 Reverse priming site

2410 TGA GTTATCTGA CTAATCTTA GTTGTATTG TCATGGTTTA ATACAATATG
ACT CAAATAGACT GATTAGAAT CAAACATAAC AGTACAAAT TATGTTATAC

FIG. 17

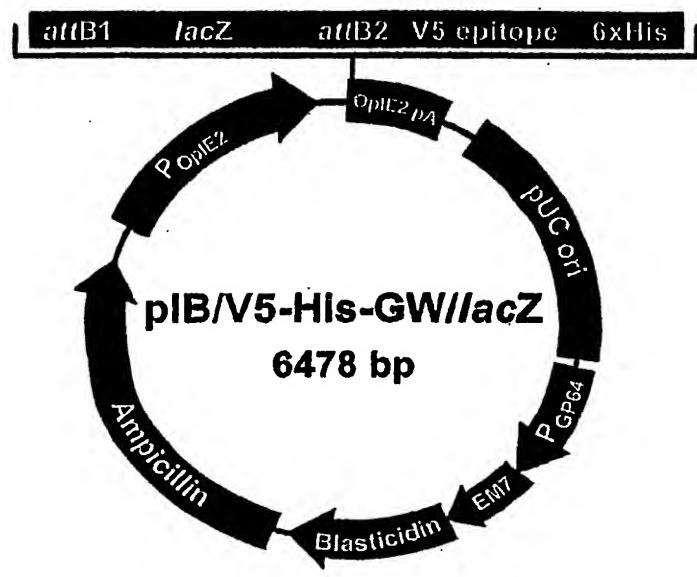


FIG. 18

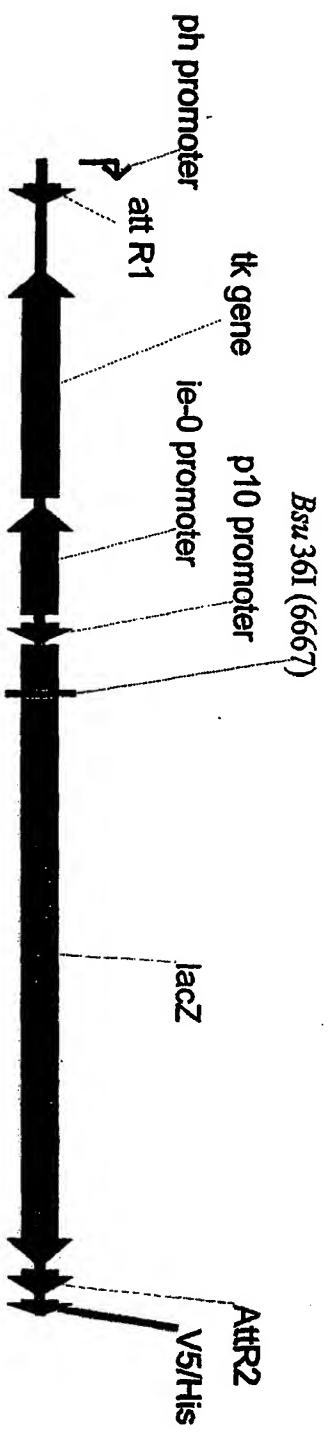


FIG. 19A

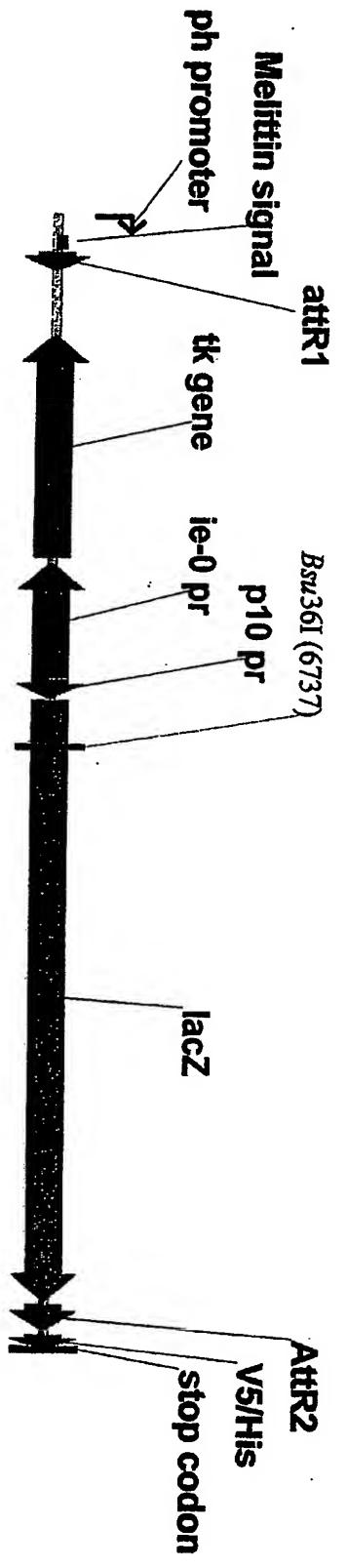


FIG. 19B

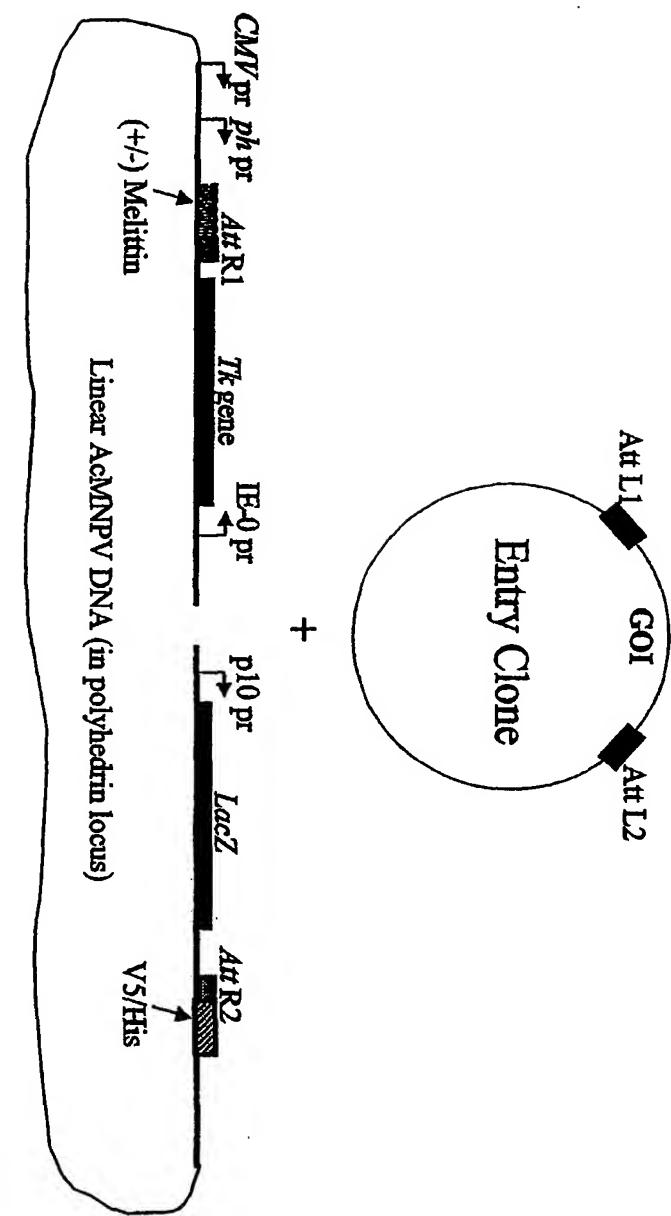
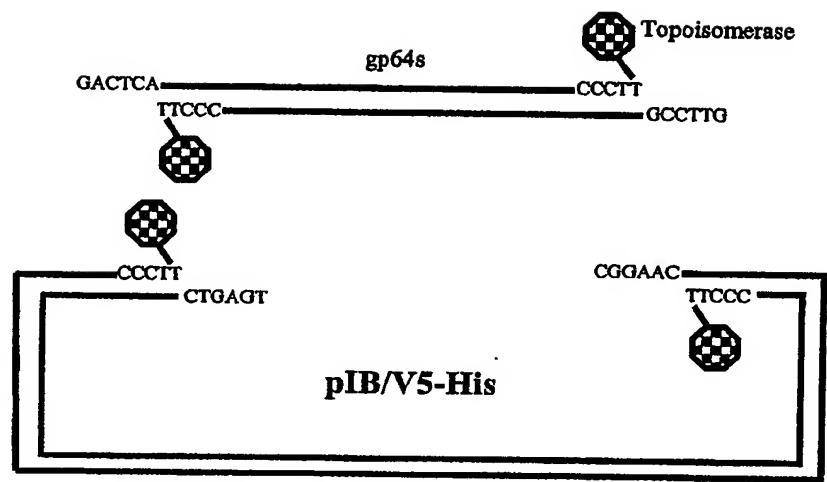


FIG. 20

FIG. 21



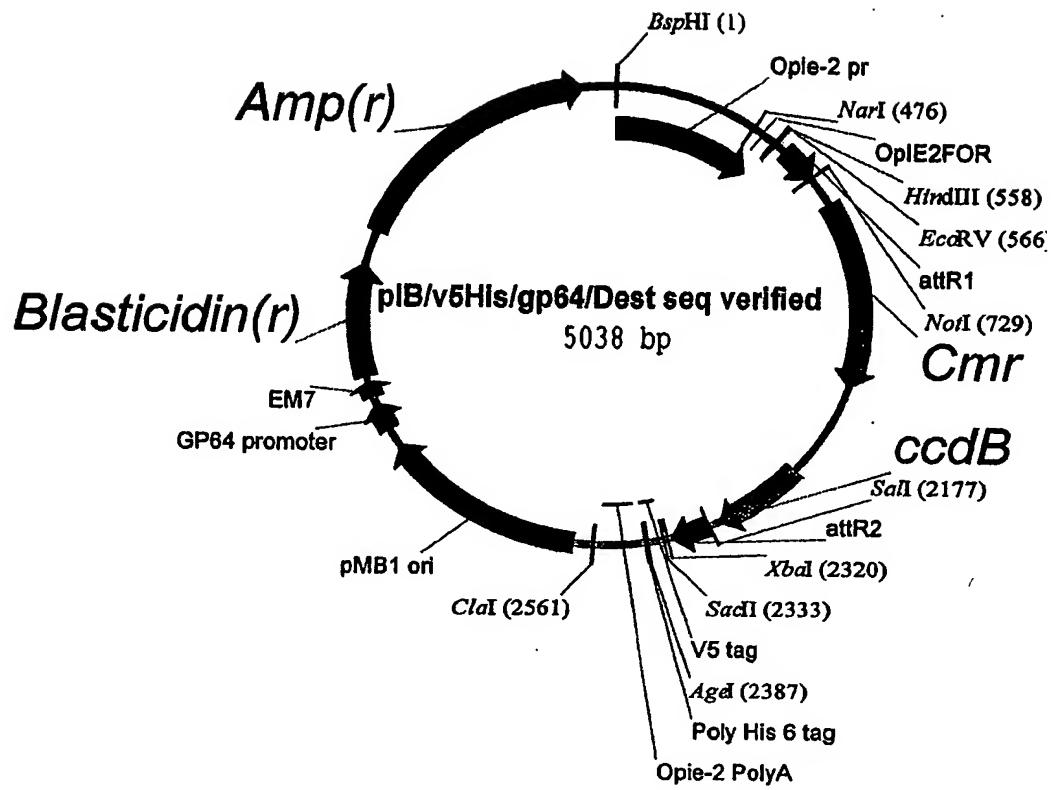


FIG. 22

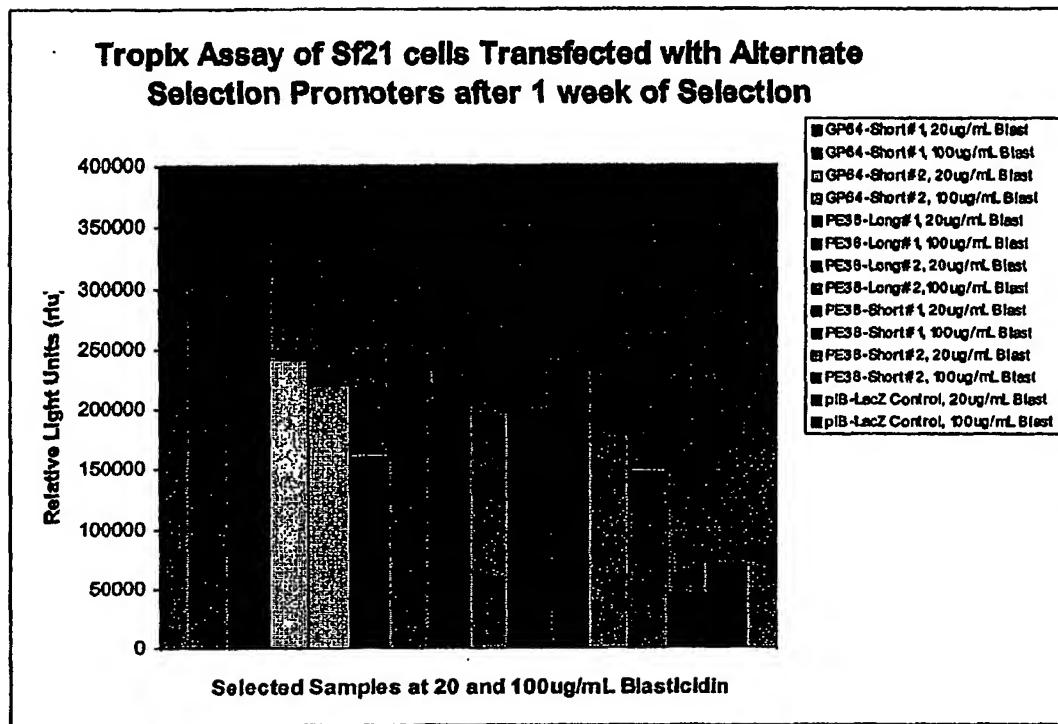


FIG. 23

FIG. 24

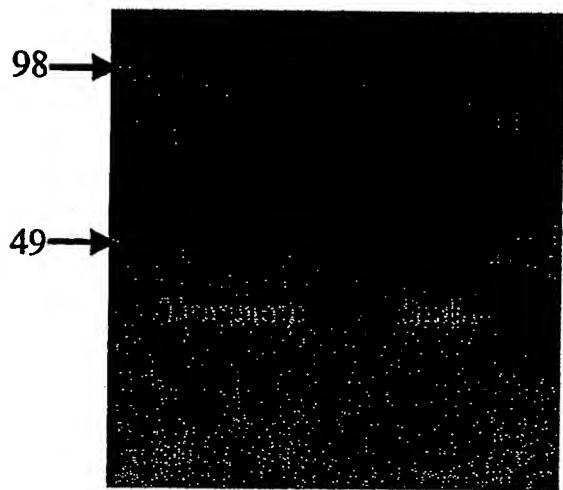
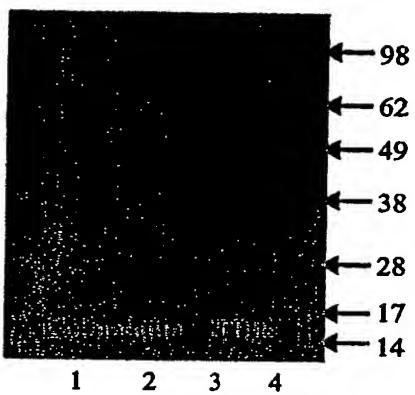


FIG. 25

A.



B.

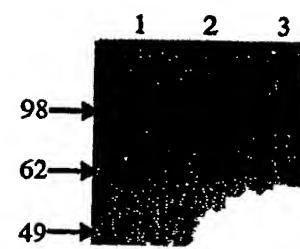


FIG. 26

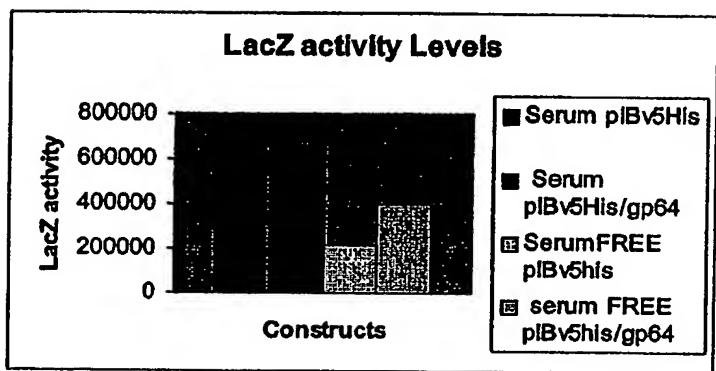


FIG. 27A

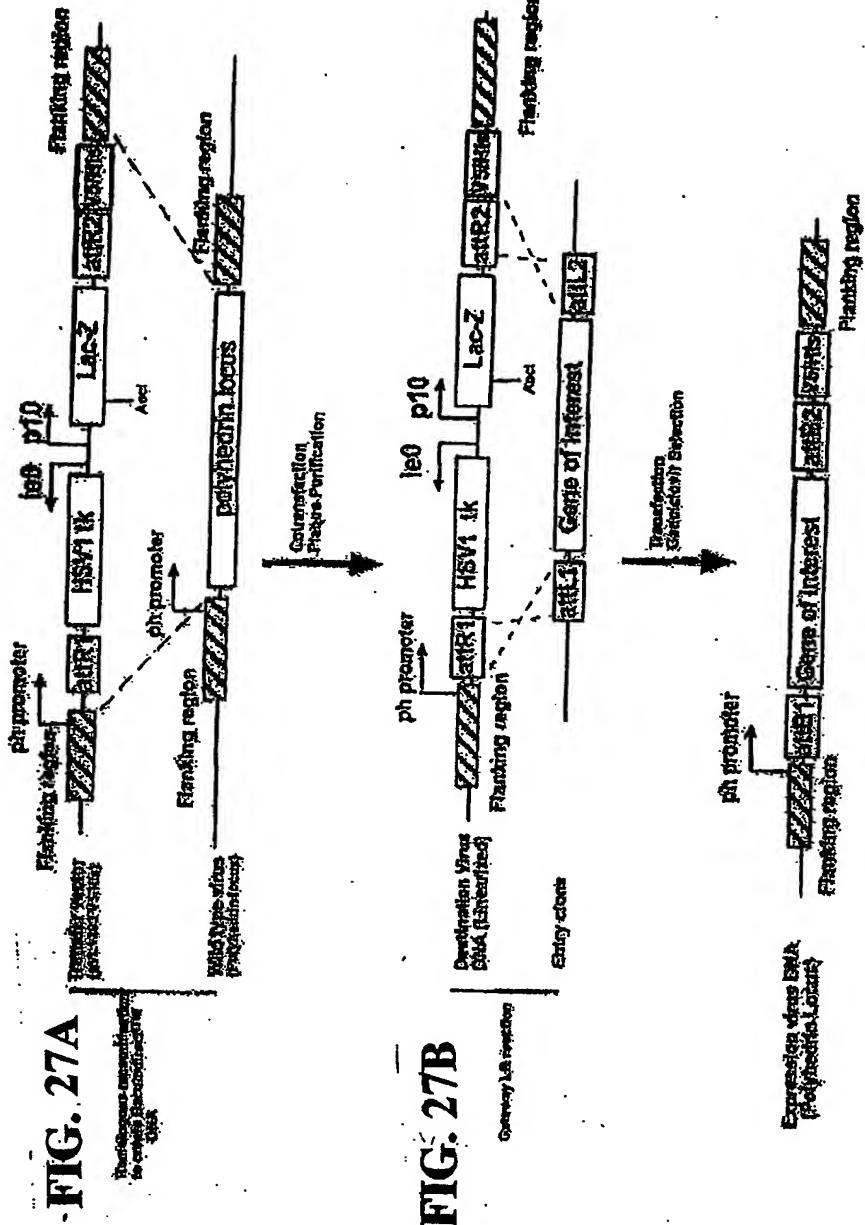
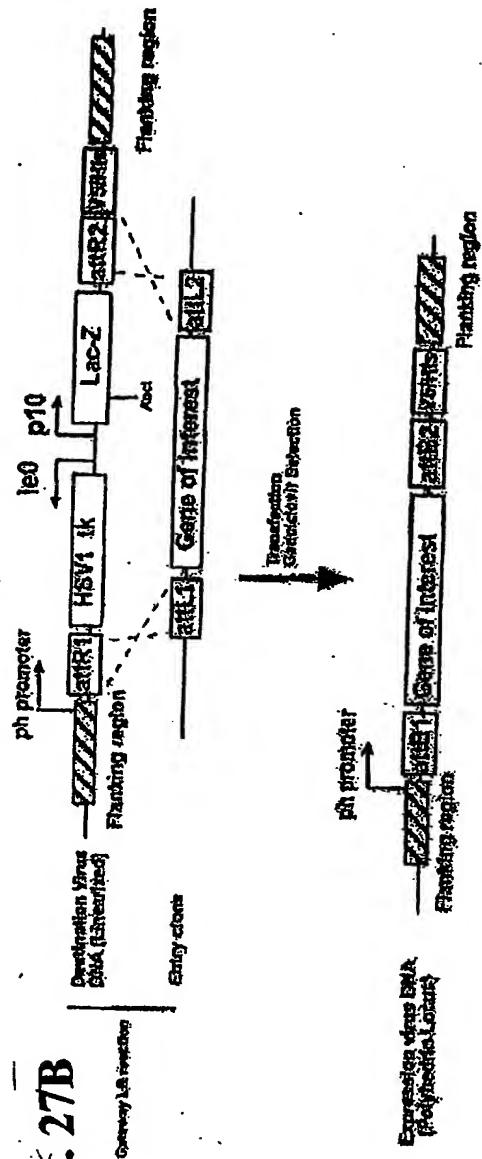


FIG. 27B



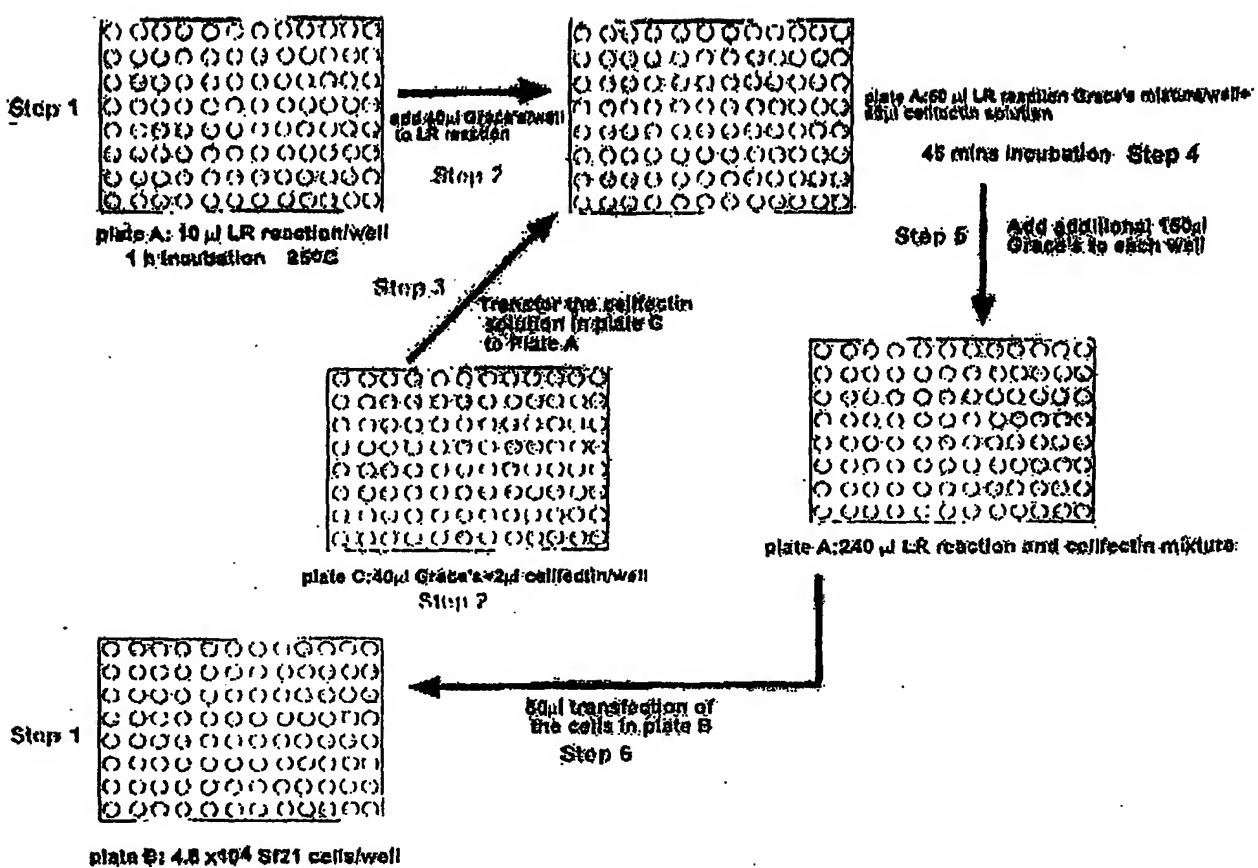


FIG. 28

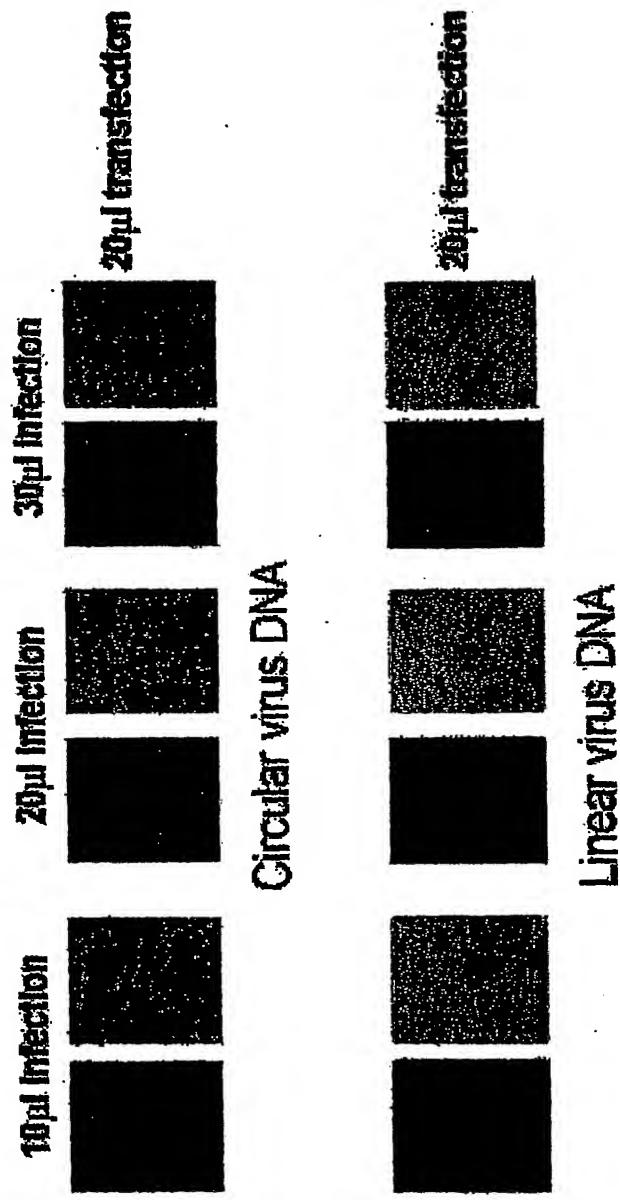
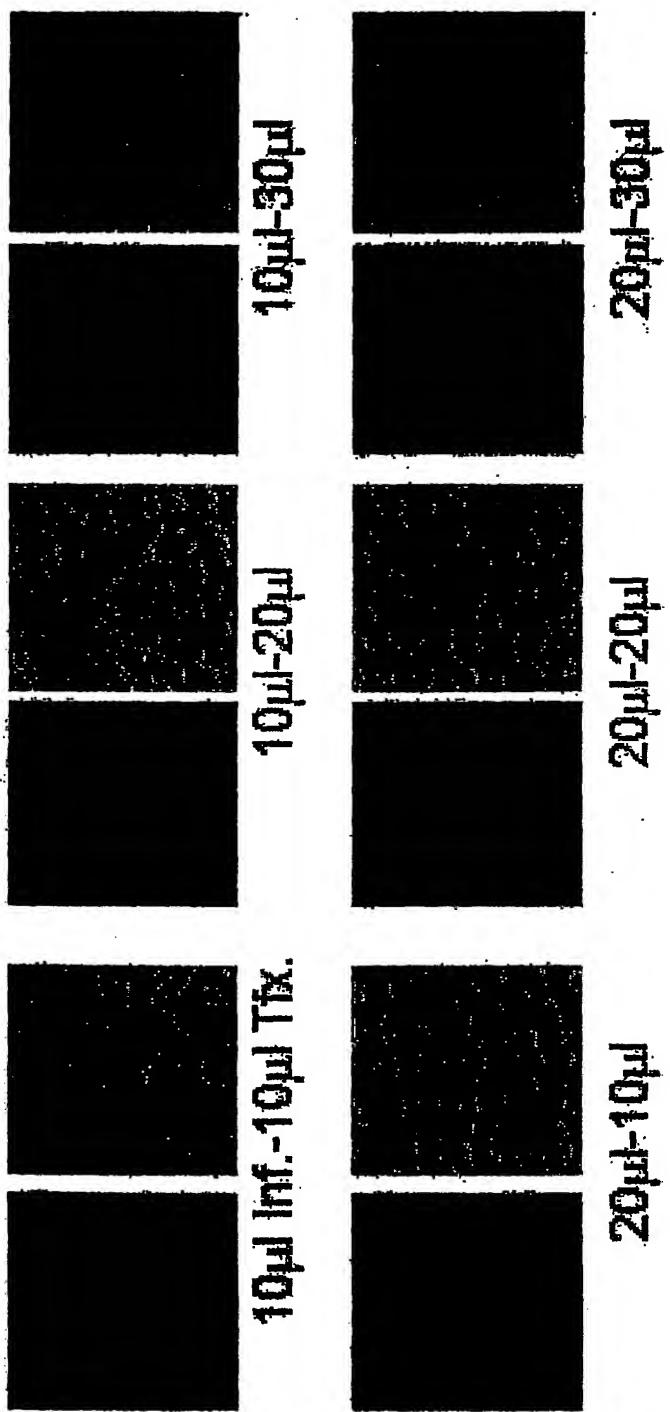


FIG. 29

FIG. 30



CAT GUS LACZ GAL APO

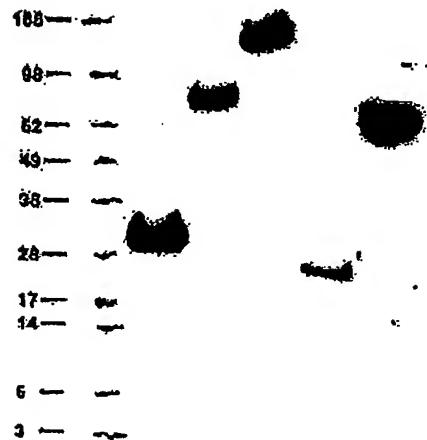


FIG. 31

Virus	Titer pfu/ml.	Transfect.	Infect.
Baculodirect™	9.8X10 ⁶	6.9X10 ⁶	
Bac to Bac	6.9X10 ⁶	7.2X10 ⁶	
MaxBac	N/A		3.6X10 ⁸

Titer comparison of the three viruses by using TCID₅₀

Virus	Titer pfu/ml.	Transfect.	Infect.
Baculodirect™	6X10 ⁶	3X10 ⁶	
Bac to Bac	8X10 ⁶	5X10 ⁶	
MaxBac	N/A		3X10 ⁸

Titer comparison of the three viruses by using plaque assay

FIG. 32

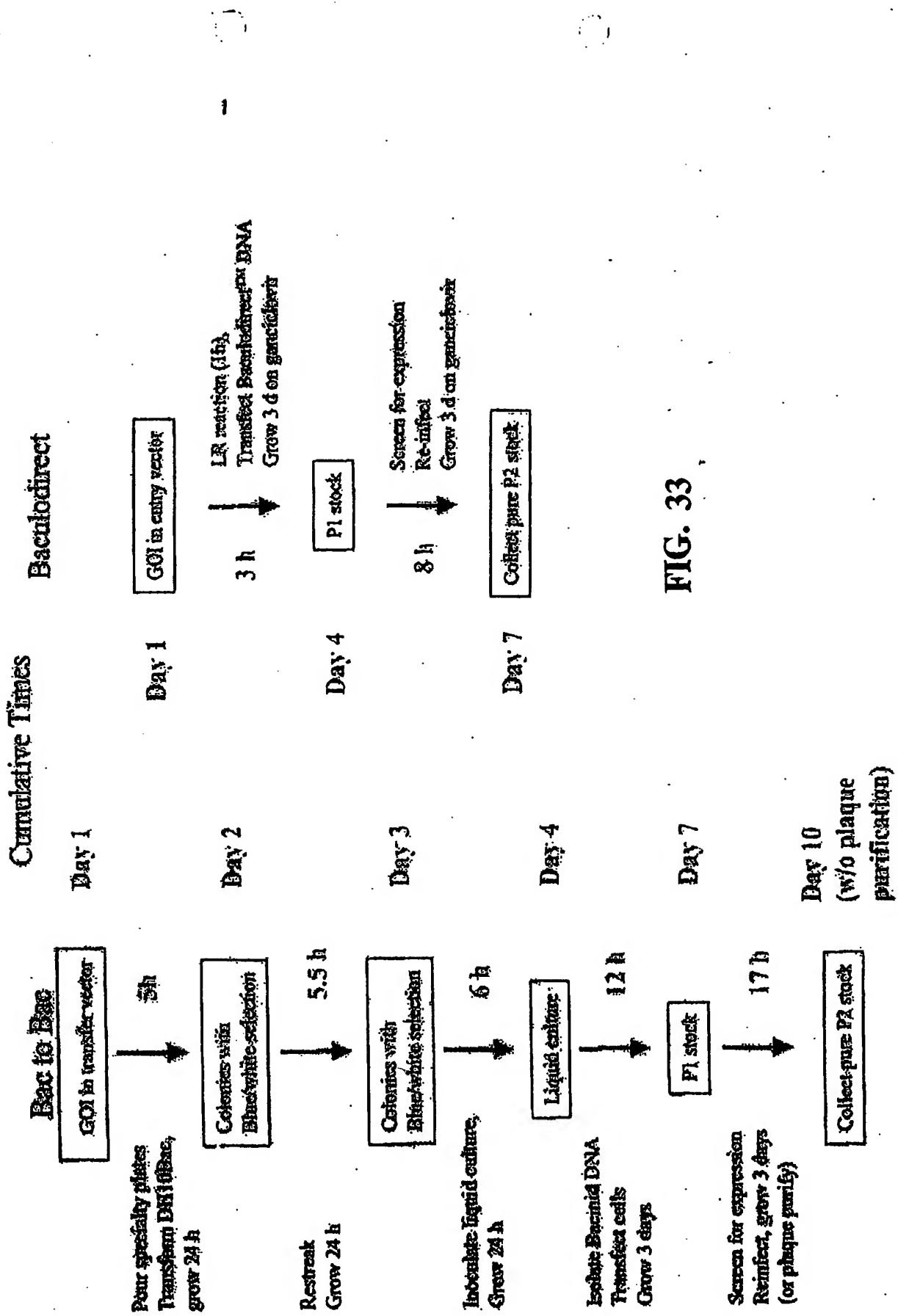


FIG. 33

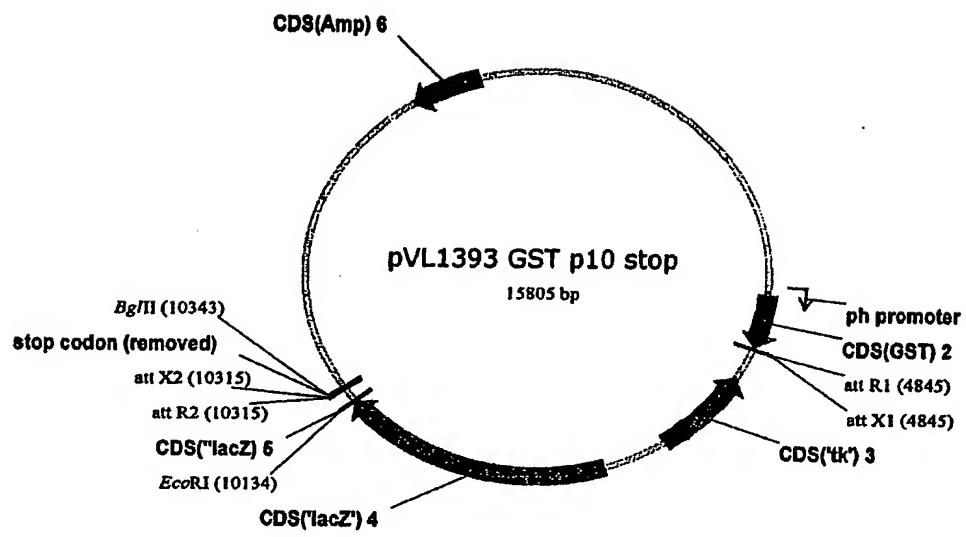


FIG. 34

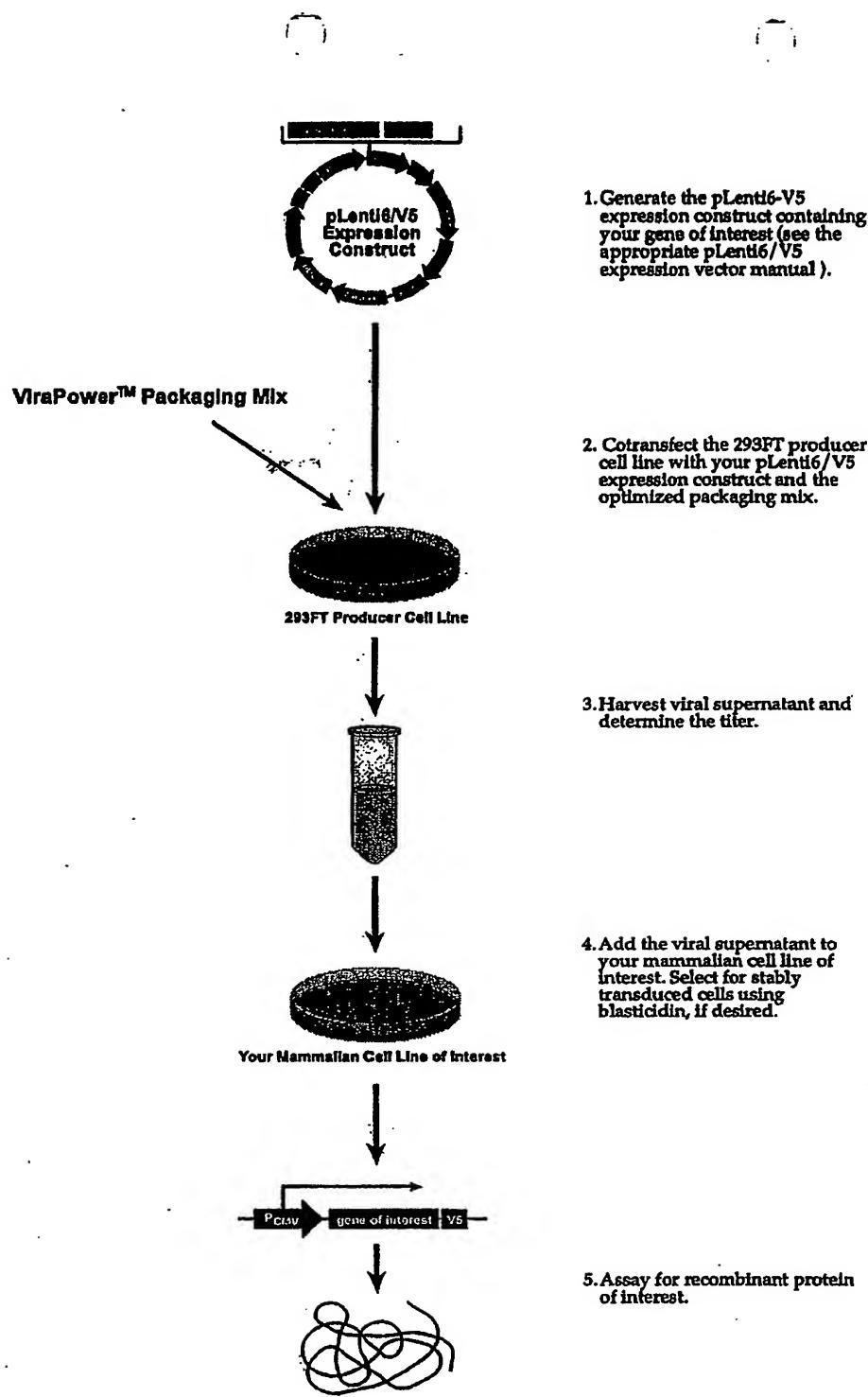


FIG. 35

FIG. 36 A

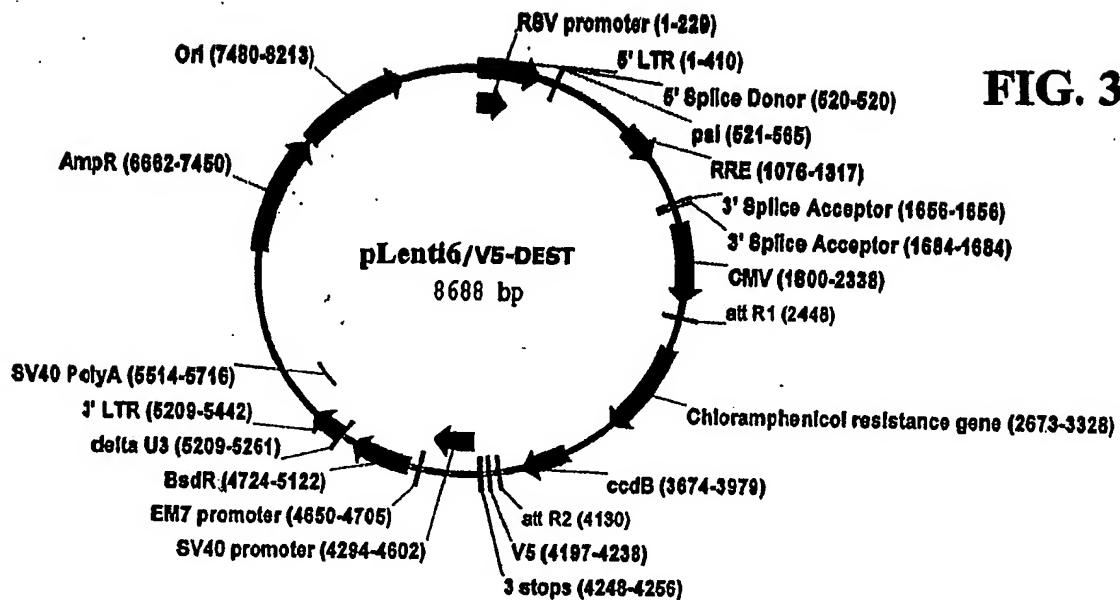
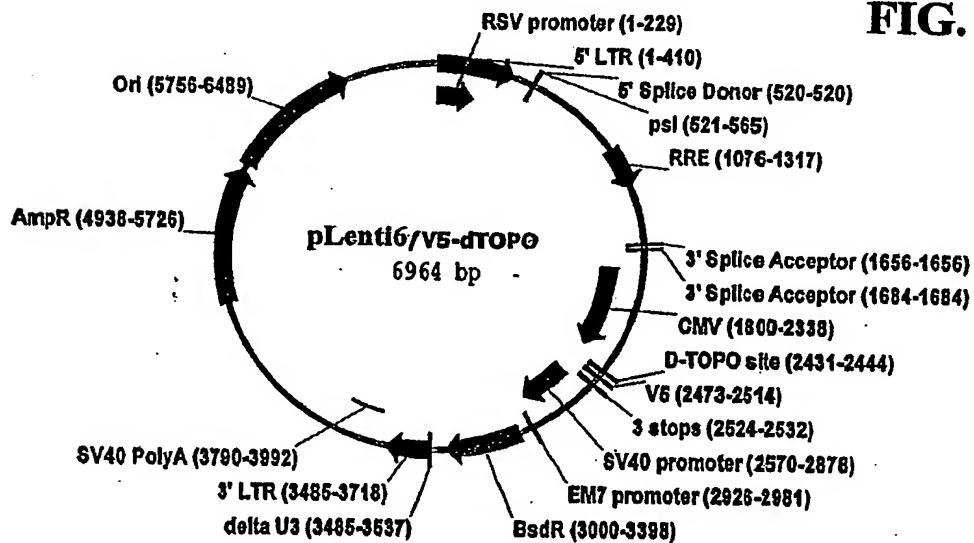


FIG. 36 B



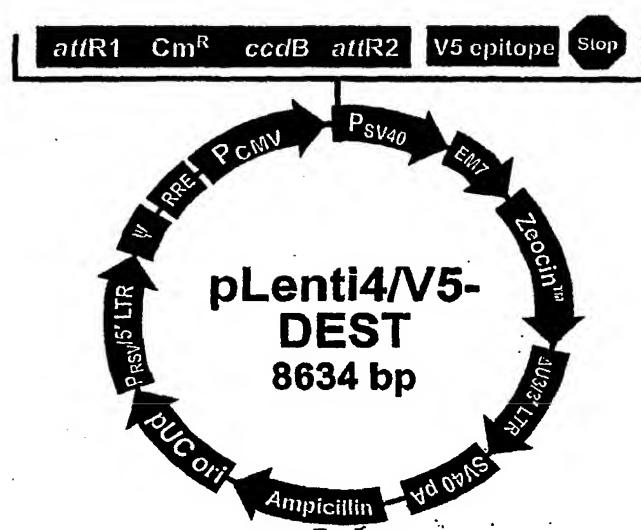


FIG. 36C

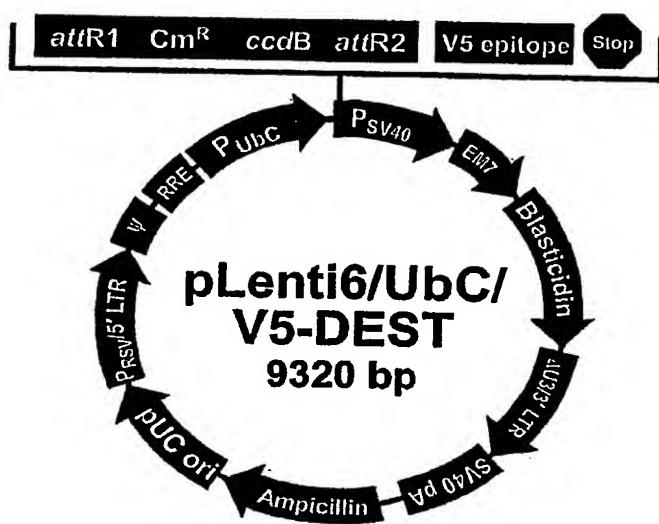


FIG. 36D

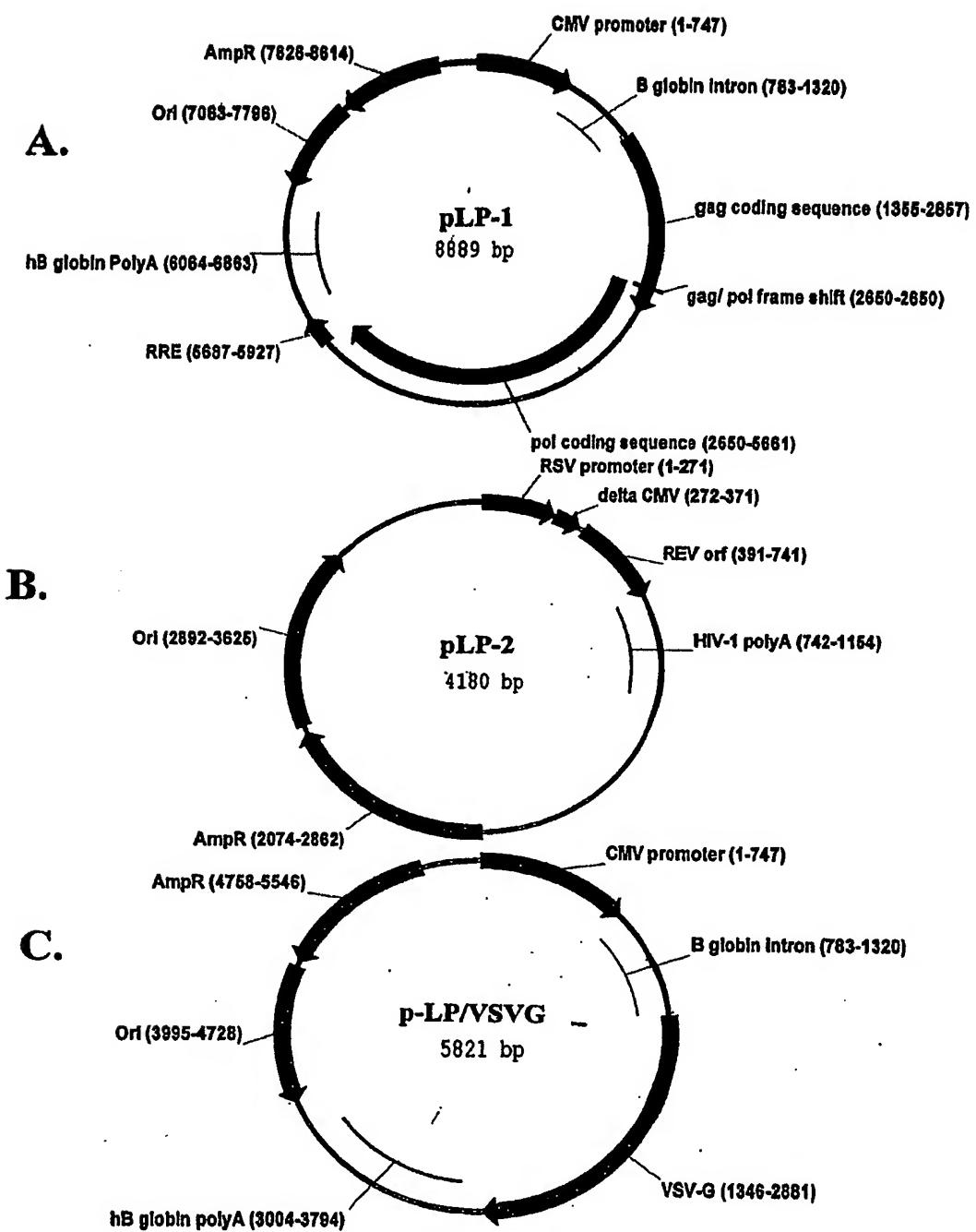
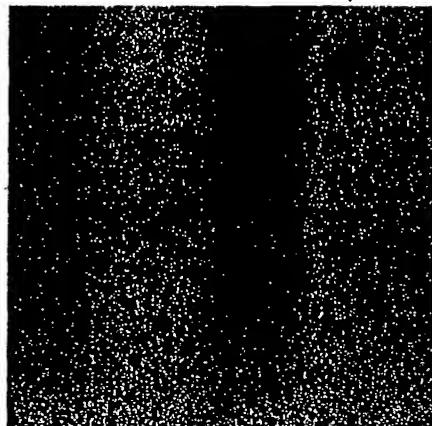


FIG. 37

A.



B.

[Bsd] in plate	LR reaction	DEST alone	DEST + CAT
No Bsd		24	320 ¹ (12/24 = 50%) ²
50 ug/ml Bsd		0	162 (24/24 = 100%) ²

¹see photo above

²percentage of correct colonies

FIG. 38

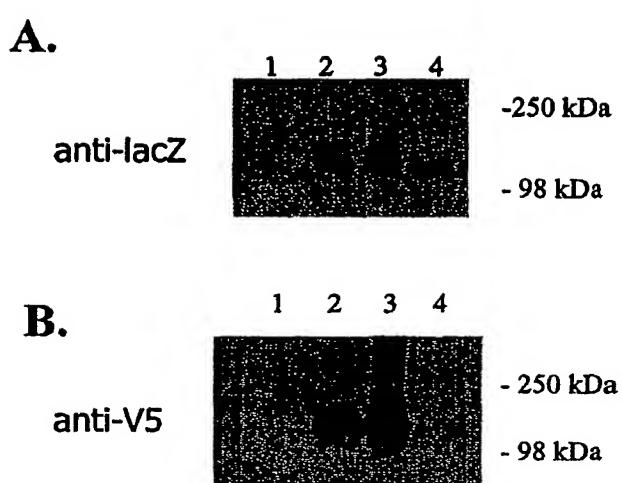


FIG. 39

Examples of Production Titers (Bsd^R cfu/ml)

	Empty	LacZ	GFP	CAT	PKC
Exp 1	6×10^6	5×10^5	4×10^6	n.d.	n.d.
Exp 2	3×10^7	3×10^5	6×10^6	8×10^6	n.d.
Exp 3	7×10^6	6×10^5	2×10^6	1×10^7	3×10^6
AVG	1.4×10^7	4.7×10^5	4×10^6	9×10^6	3×10^6

n.d. = not determined

FIG. 40

A.



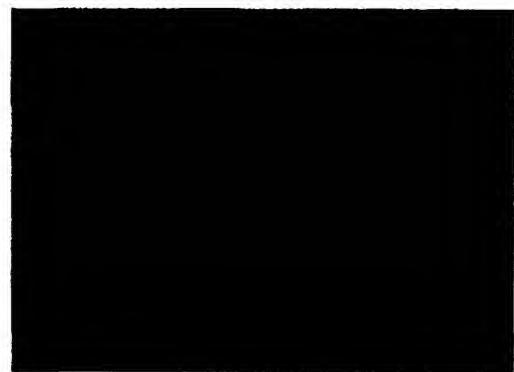
pLenti6/V5-GW/lacZ

B.



Brightfield

C.



Fluorescent

pLenti6/V5-dT/GFP

FIG. 41

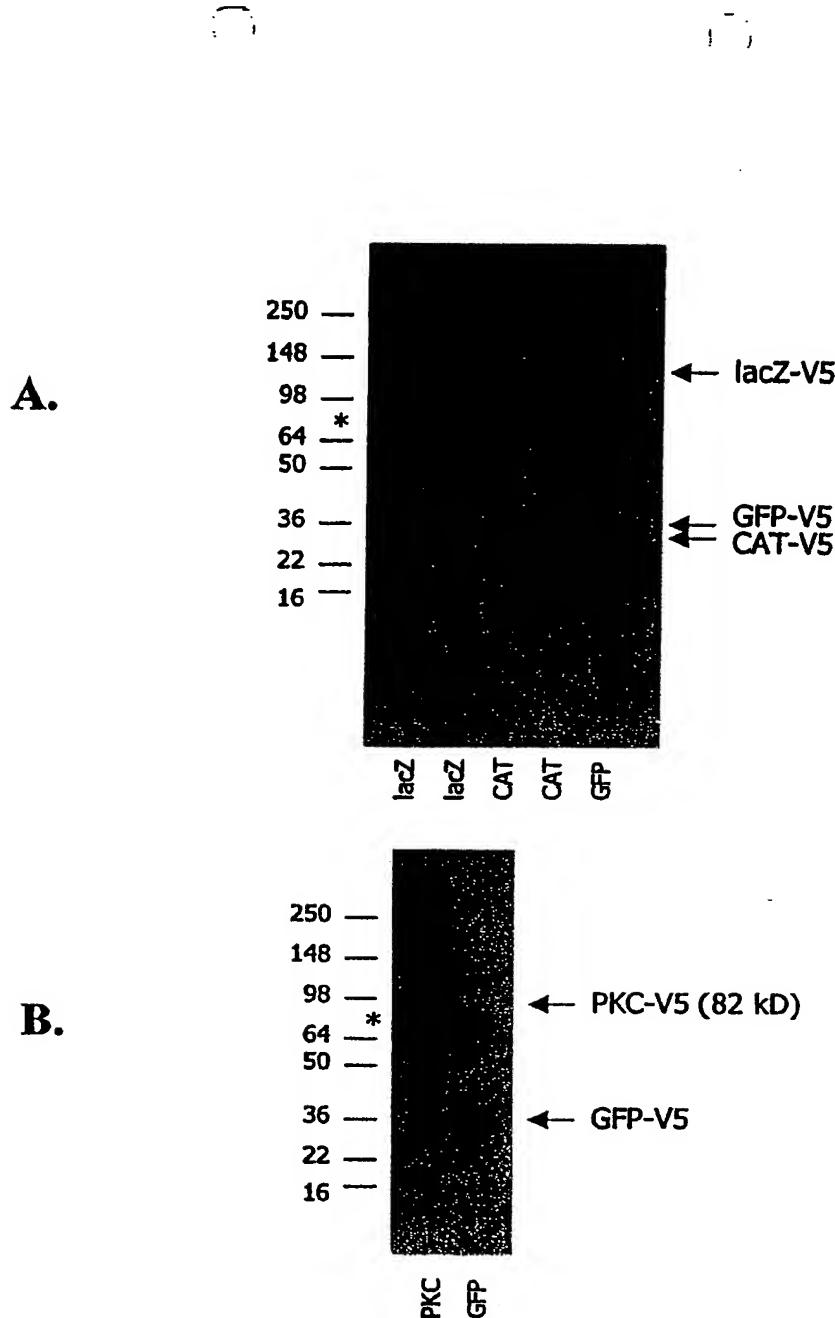


FIG. 42

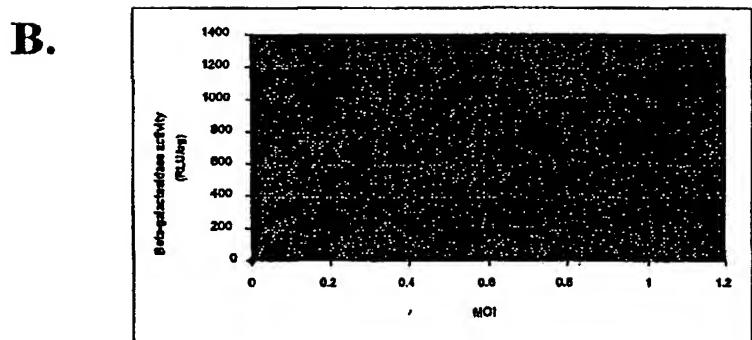
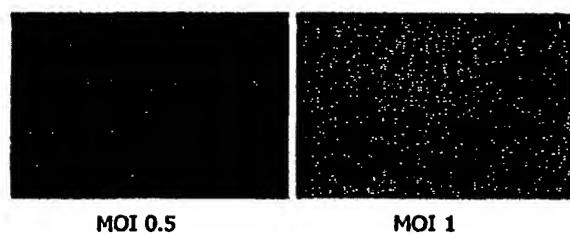
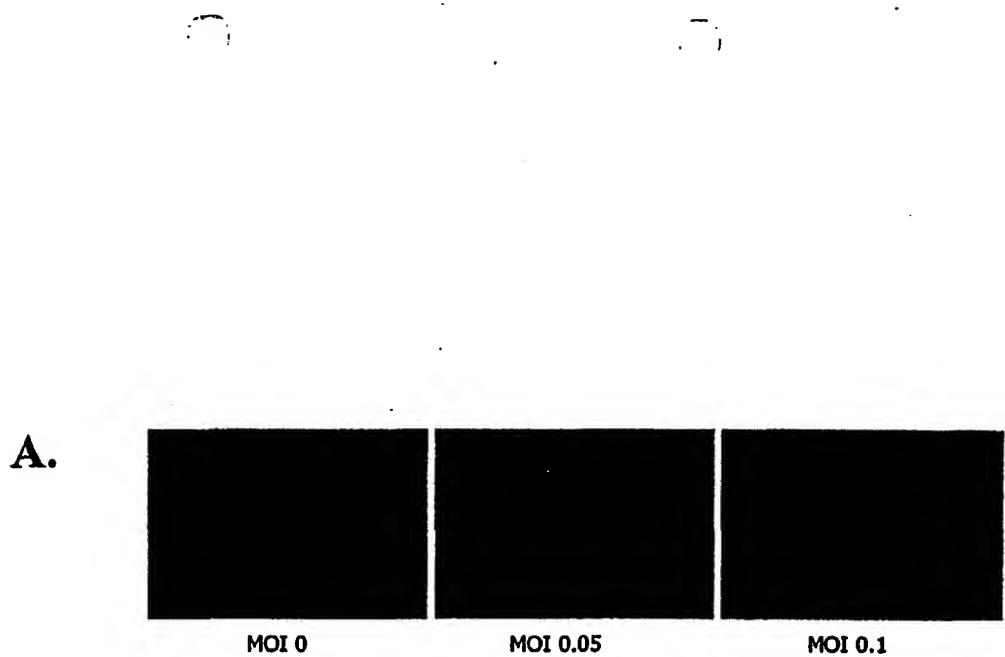
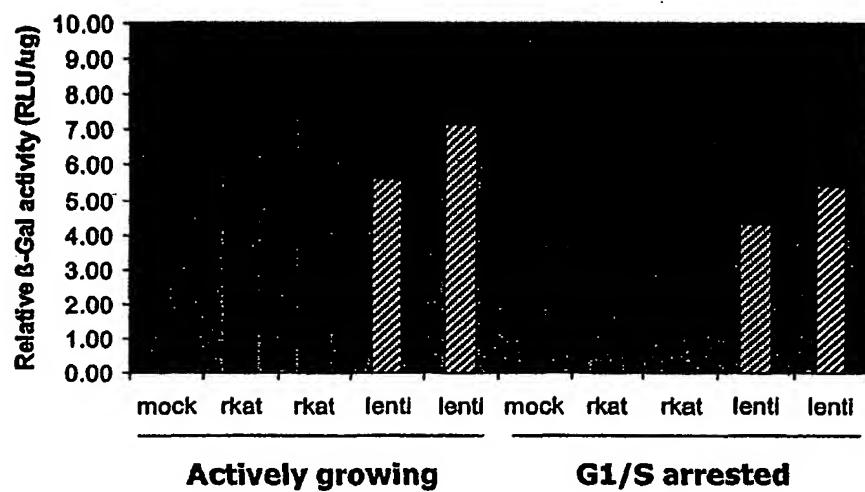


FIG. 43

A.



B.

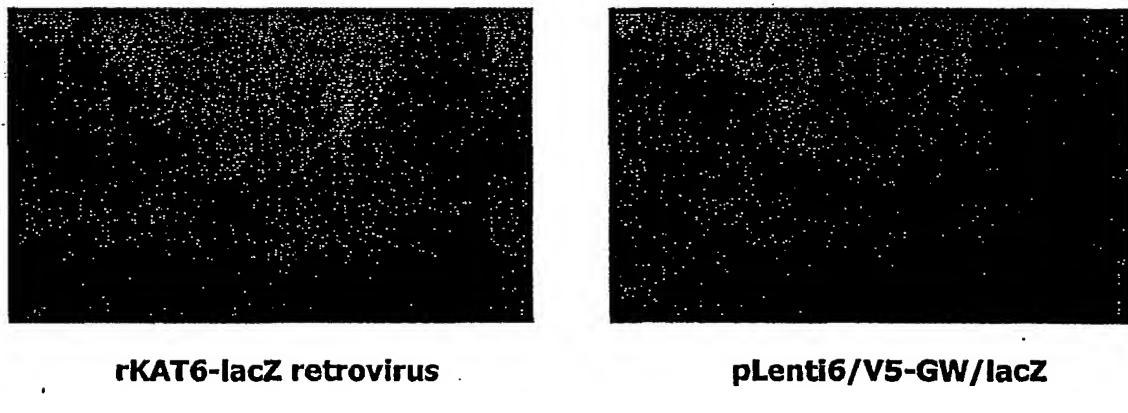


FIG. 44

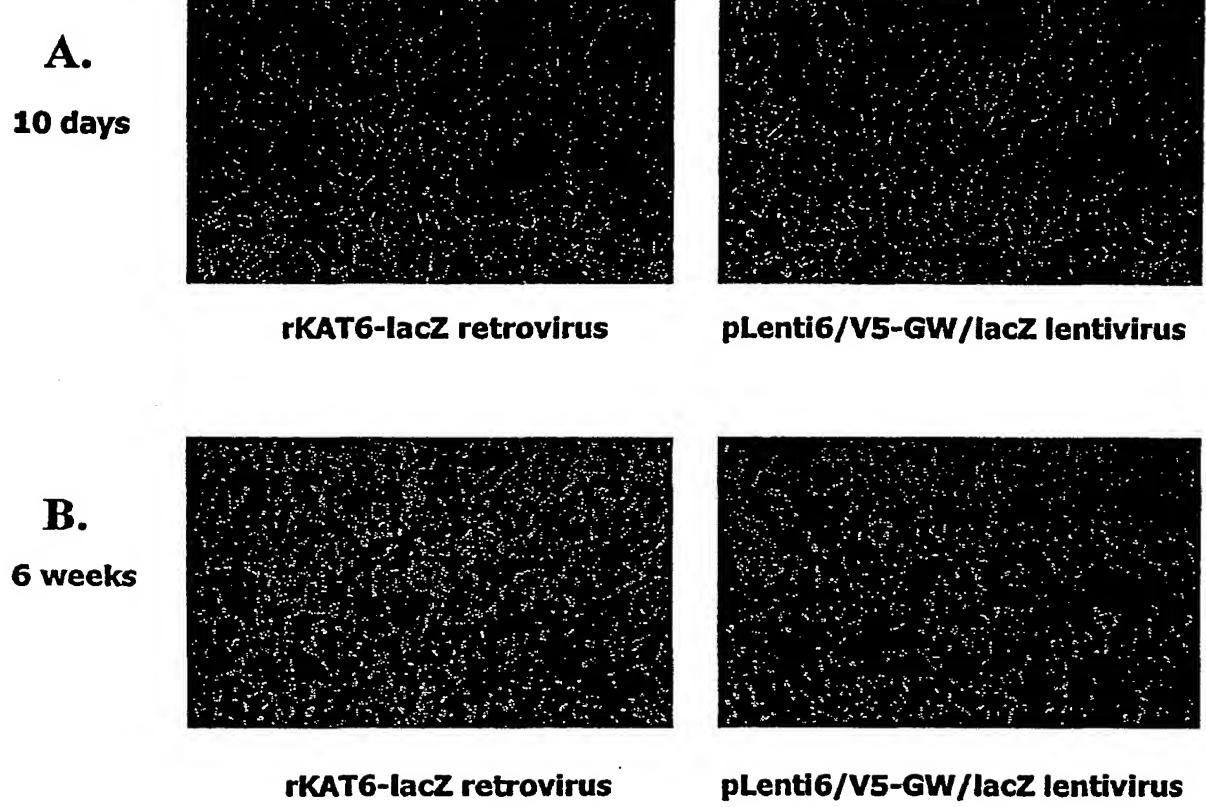


FIG. 45

FIG. 46A

CAAT CMV forward priming site TATA

2251 TCGTACCAAC TCCGCCCAT TGACGCAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTCGTTAG

Transcriptional start

2331 TGAACCGTCA GATCGCCTGG AGACGCCATC CACGCTGTT TGACCTCCAT AGAAGACACC GACTCTAGAG GATCCACTAG

2411 TCCAGTGTGG TGGAATTCTG CAGATATCAA CAAGTTT GTCTATAGTT GTTCAAACAT GTT

2448 ... Pro Ala Phe Leu 4130
aEB1 aEB2

4134 Tyr Lys Val Val Asp Ile Gln His Ser Gly Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu GLY
TAC AAA GTG GTT GAT ATC CAG CAC AGT GGC GGC CGC TCG AGT CTA GAG GGC CCG CGG TTC GAA GGT
TTT CAC CAA CTA TAG GTC GTG TCA CCG CCG GCG AGC TCA GAT CTC CCG GGC GCC ARG CTT CCA

4200 Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly *** *** ***
AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTT
TTC GGA TAG GGA TTC GGA GAG GAG CCA GAG CTA AGA TGC GCA TGG CCA ATC ATT ACT

V5 epitope V5 (C-term) reverse priming site

FIG. 46B

US forward priming site

2881 TTGGCGAGTC TGTTTGTGA AGTTTTTAG GCACCTTTG AAATGTRATC ATTTGGCTCA ATATGTAATT TTCACTGTTA

2961 GACTAGTAA TTGTCGCTA AATTCTGGCC GTTTTGCTAG ACGAAGCTTG GTACCGAGCT CGGATCCACT

3041 AGTCCAGTGT GGTGGAATTG TGCAAGATATC ACAAGTTT
ACGTCTATAG TTGTTCAAAC ATGTTT

3079 ... Pro Ala Phe 4762

Leu Tyr Lys Val Val Asp Ile Gln His Ser Gly Arg Ser Ser Leu Glu Gly Pro Arg Phe Glu
4763 TTG TAC AAA GTG GTT GAT ATC CAG CAC AGT GGC GGC CGC CGC TCG AGT CTA GAG GGC CGG CGG TTC GAA
[REDACTED] TTT CAC CAA CTA TAG GTC GTG TCA CCG CCG GCG AGC TCA GAT CTC CCG GGC GCC AAG CTT

Gly Lys Pro Ile Pro Asn Pro Leu Leu Asp Ser Thr Arg Thr Gly *** *** ***
4829 GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTT
CCA TTC GGA TAG GGA TTC GGA GAG CCA GAG CTA AGA TGC GCA TGG CCA

V5 epitope V5 (C-term) reverse priming site

FIG. 46C

1786 ^{5' end of U6 promoter}
 CCGATCTGGC CTCCGCGCGG GGTTTGGCG CCTCCCGCGG GCGCCCCCT CCTCACGGCG AGCGCTGCCA CGTCAGACGA

 1876 AGGGCGCAGG AGCGTCTGA TCTTSp 1 GCGCGCTCA GGACAGCGGC CGCGCTCTCA TAAGACTCGG CCTTAGAAC

 1956 CCAGTATCAG CAGRAGGACA TTTAGGACG GGACTGGGT GACTCTAGGG CACTGGTTT CTTCCAGAG AGCGGAACAG

 2036 GCGAGAAAAA GTAGTCCCTT CTGGGGATT CTGGGGAGGG ATCTCGTSp 1 TATA box GCGCGTGAC GCGGATGATT ATATAAGGAC
 → Start of Transcription

 2116 GCGCCGGTG TGGCACAGCT AGTCCGTCC CAGCCGGAT Exon 1 TGGGTCGG GTTCTGTT GTTGATCGCT GTGATCGCA

 2186 ^{5' end of Intron 1}
CTTGCTGAGT AGGGGGCTGC TGGGCTGGCC GGGGCTTTCG TGGCGCCCGG GCGCTCGGT GGGACGGAGC CGTGTGGAGA

 2276 GACCGCCAAG GGCCTGAGTC TGGGTCGGCG AGCAAGGTTG CCCTGAACG GGGGTTGGGG GGAGCGCAGC AAAATGGCGG

 2356 CTGTTCCCGA GTCTGAAATG GAAGACGCTT GTGAGGGGGG CTGTGAGGTC GTGAAACAA GGTGGGGGGC ATGGTGGGGC

 2436 GCAAGAACCC AAGGTCTTGA GGCCTCGCT AATCGGGGA AGCTCTTATT CGGGTGAGAT GGGCTGGGC ACCATCTGGG

 2516 GACCCCTGACG TGAGTTTGT CACTGACTGG AGAACTCGGT TTGTCGTCTG TTGGGGGGC GGCAGTTATG CGGTGCCGTT

 2596 GGGCAGTCGA CCGTACCTT TGGGAGCGCG CGCCCTCGTC GTGTCGTAC GTCAACCGTT CTGTCGCTT ATAATGCAGG

 2676 GTGGGGCCAC CTGGGGTAG GTGTCGGTA GCCTTTCTC CGTCCACGA CGCAGGGTC GGGCTAGGG TAGGCTCTCC

 2756 TGAATCGACA GGGCGGGAC CTCTGGTGAG GGGAGGGATA ACTGAGGCGT CAGTTCTTT GGTCGGTTT ATGTAACCTAT

 2836 CTTCTTAAGT AGCTGAGGCT CGGGTTTGA ACTATGCGCT CGGGTTGGC GAGTGTGTTT TGTCAGTTT TTAGGCACC

 2916 TTTGAAATG TAATCATTTG GGTCAATATG TAATTTTCAG TGTTAGACTA GTAATGTC CGCTAAATTC TGGCGTTT

 2996 ^{3' end of Intron 1}
 TGGCTTTTTT GTTAGACGAA CGCTGG...
^{5' end of Exon 2}

FIG. 47

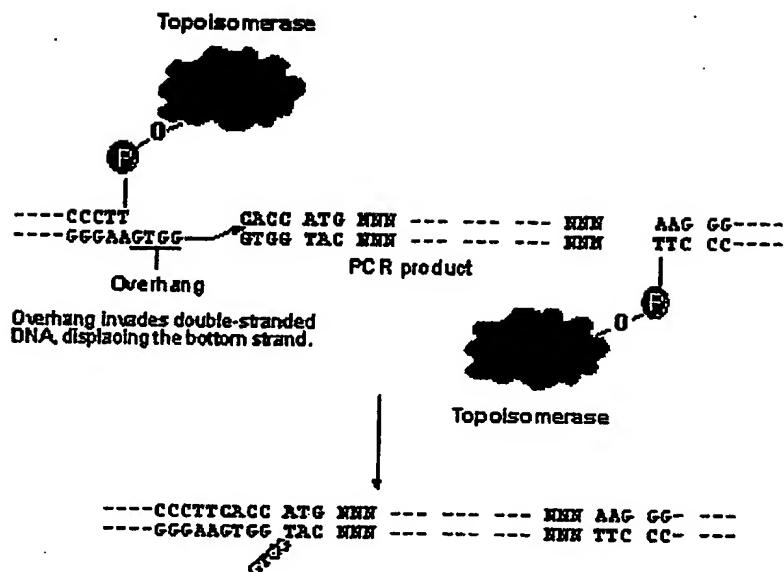


FIG. 48

CAAT CMV forward priming site

TATA 3' end of CMV promoter

Transcriptional start BamHI SpeI

2251 TCGTAACAC TCCCCCAT TGACGAAAT GGGCGGTAGG CGTGTACGGT GGGAGGTCTA TATAAGCAGA GCTCGTTAG

2331 TGAACCGTCA GATGCCCTGG AGACGCCATC CACGCTGTT TGACCTCCAT AGAAGACACC GACTCTAGAG GATCCACTAG

BsrXI XbaI ApaI SacII SstI

2411 TCCAGTGTGG TGGAAATTGAT CCCTTC ACC ATG ... AAG GGC TCG AGT CTA GAG GGC CCG CGG TTC GAA GGT
 CTA GGGAAAG TGT TAC ... TTC CCA GTT CTG
 Lys Gly Ser Ser Leu Glu Gly Pro Arg Phe Glu Gly

V5 epitope V5(C-term) reverse priming site

2476 AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTTGGAA
 Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Arg Thr Gly *** *** ***

2541 TTAATTCTGT

FIG. 49



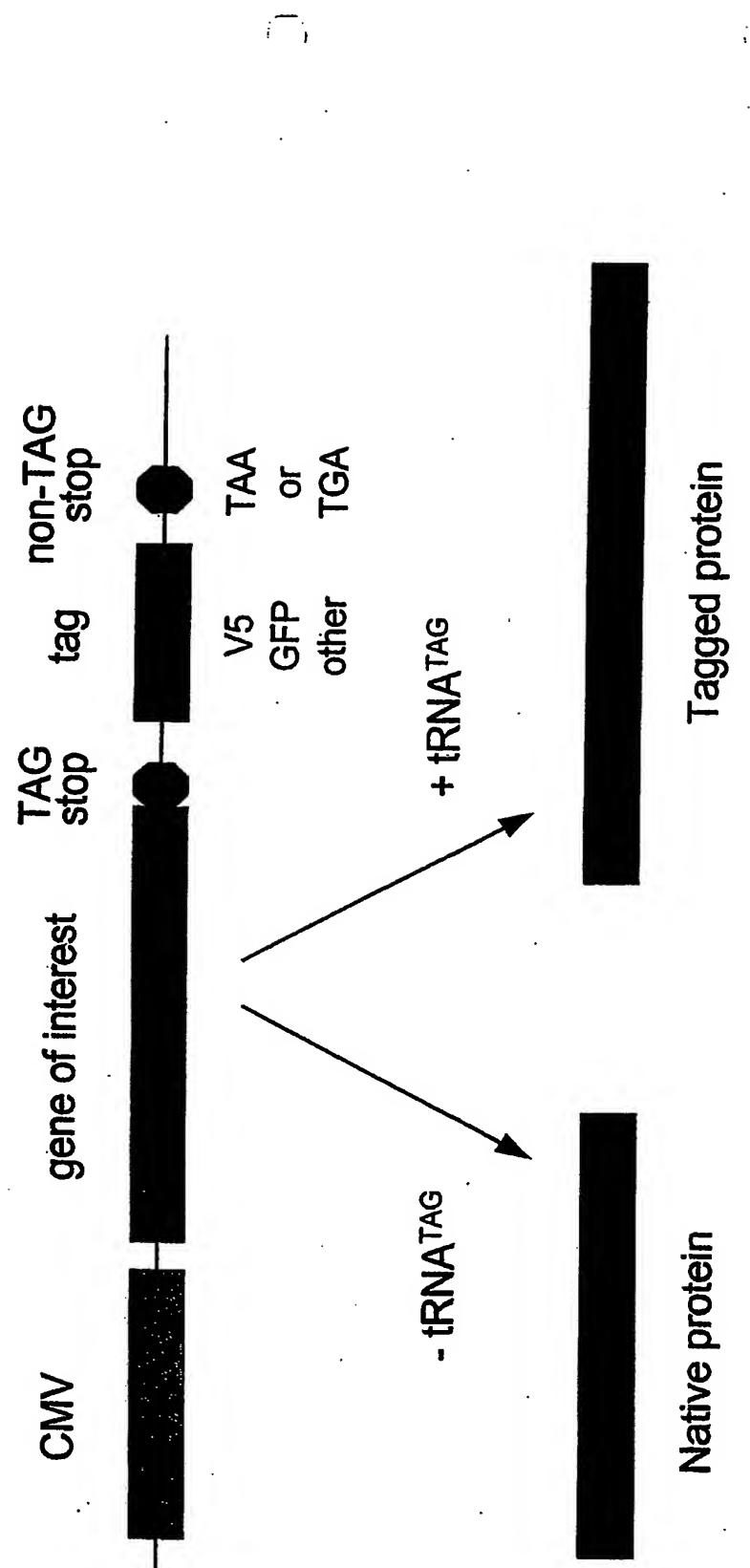


FIG. 50

FIG. 51A

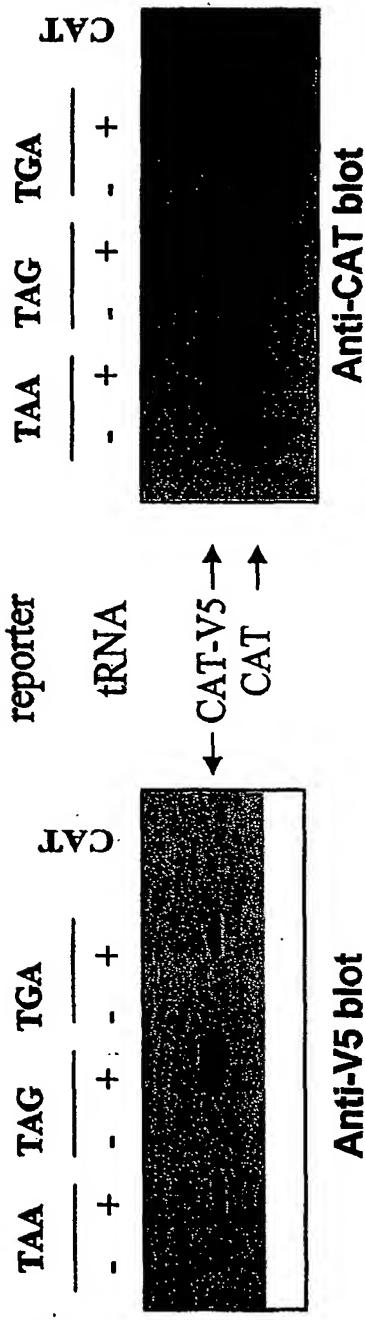
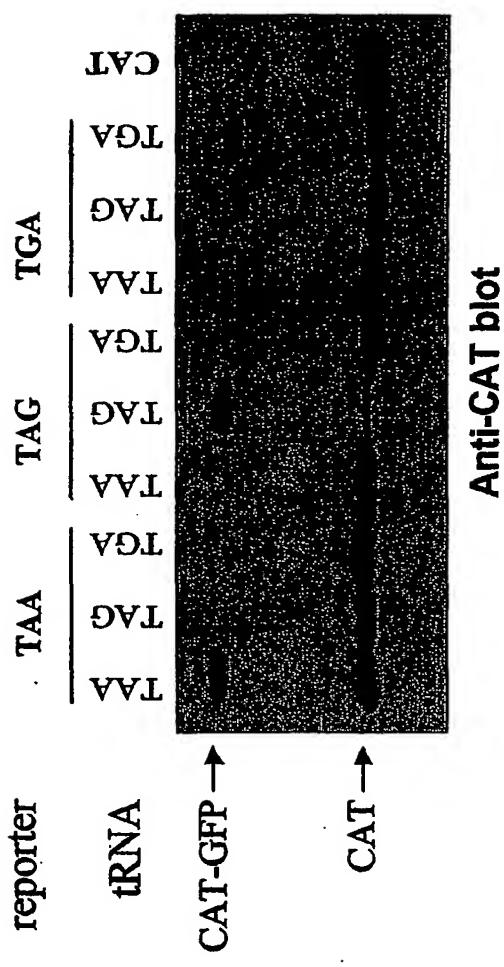
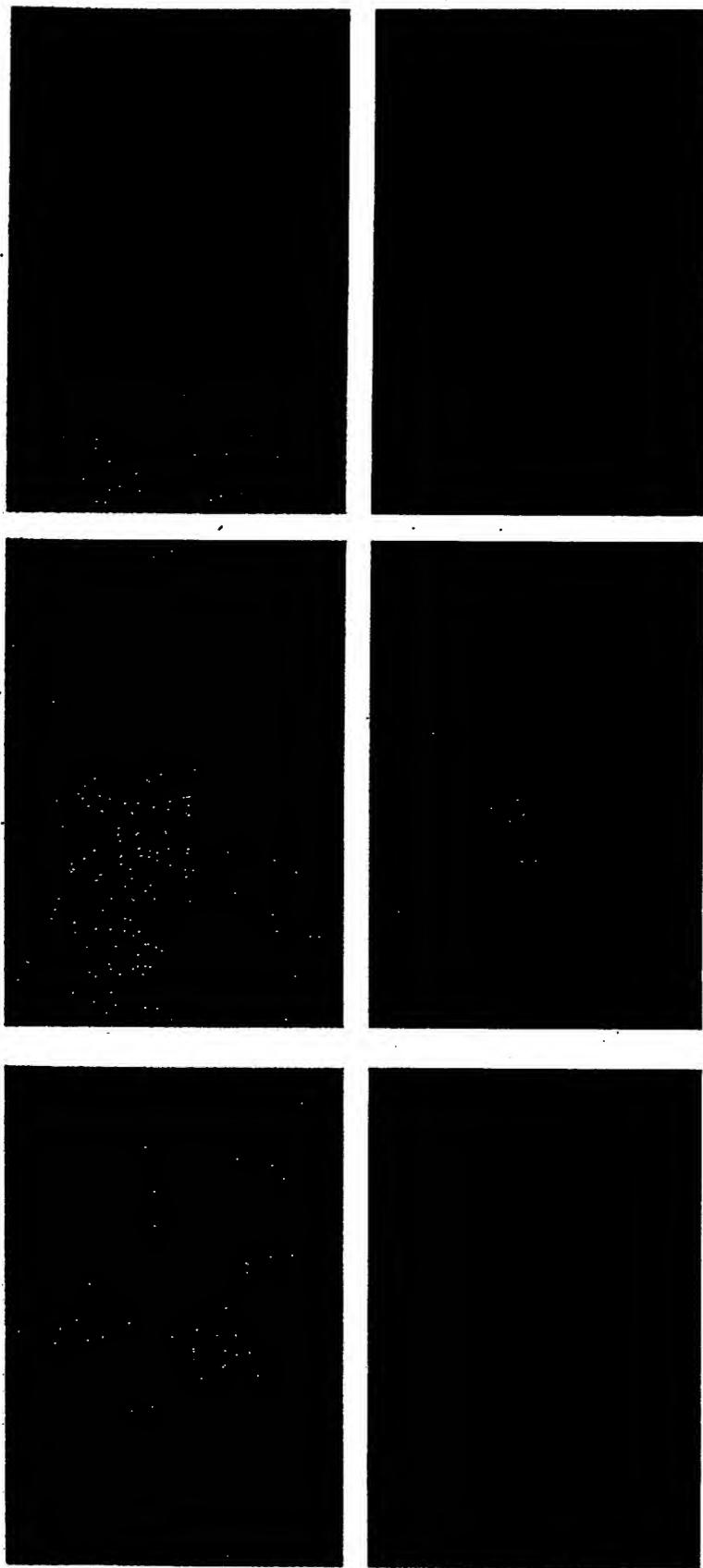


FIG. 51B



FIGS. 51 A-B



tRNA^{TAG}
tRNA^{TAA}

pcDNA3.1 lacZ-stop^{TAG}-GFP

FIG. 52

Monomer

8-mer

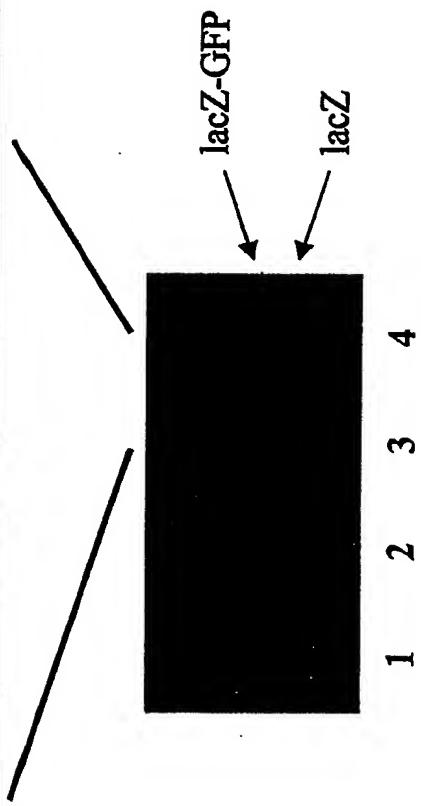
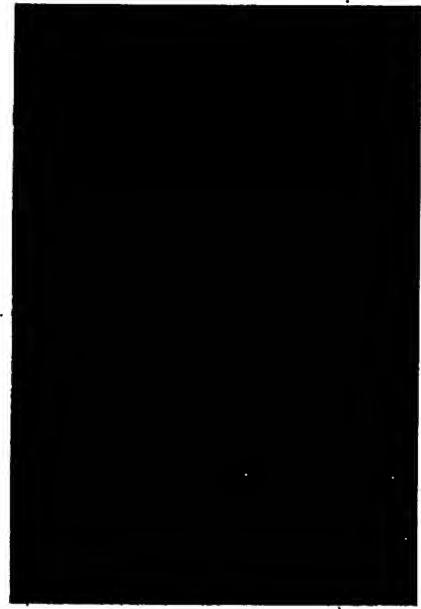
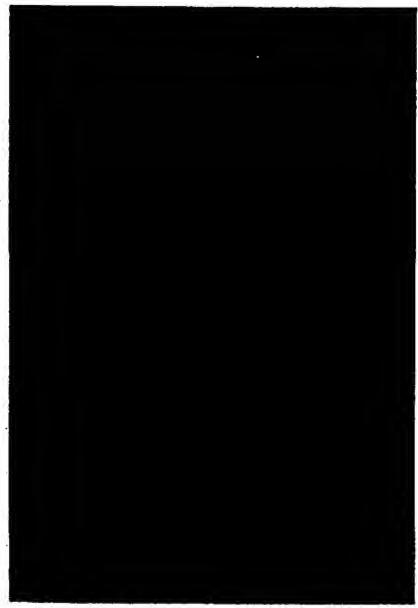


FIG. 53

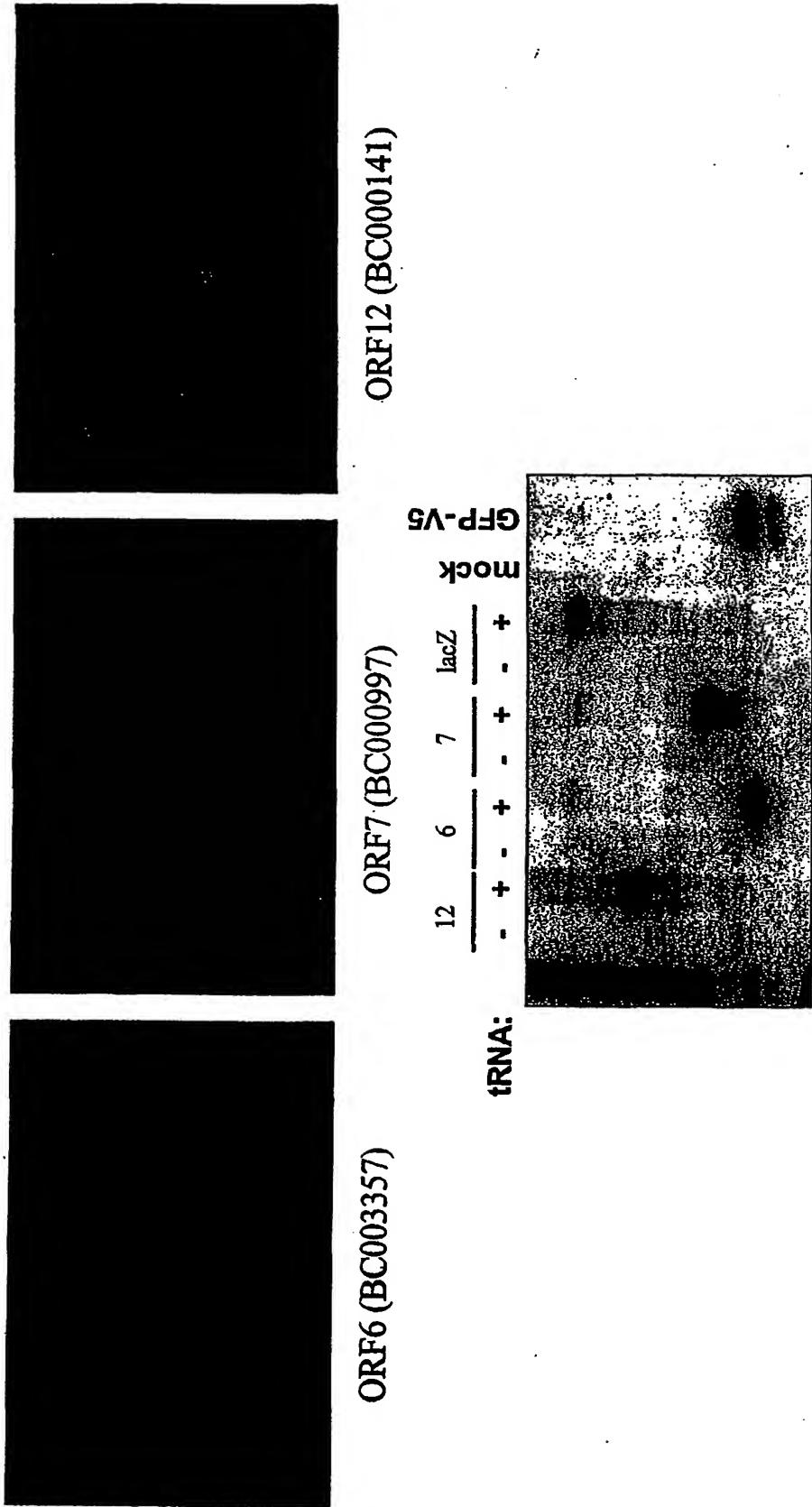


FIG. 54

FIG. 55A

MOI: 19 51 77 192

*

% suppression: 13 30 51 60

STABLE GOI

TRANSIENT GOI

MOI: 19 38 77 192

This image consists of a black rectangular frame containing a dense, scattered pattern of small white dots. The dots are irregularly spaced and have varying intensities, creating a noise-like or textured appearance. There is no discernible text or other graphical elements.

% suppression: 63 71 76 75

FIG. 55B

1

FIG. 56

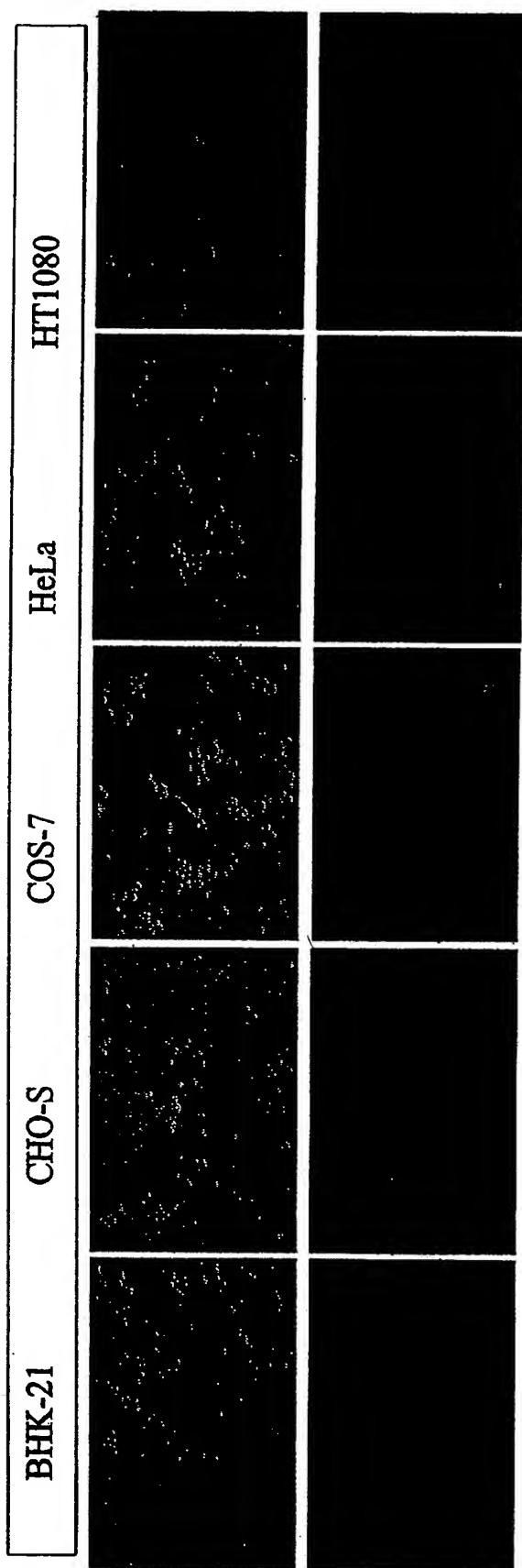


FIG. 57

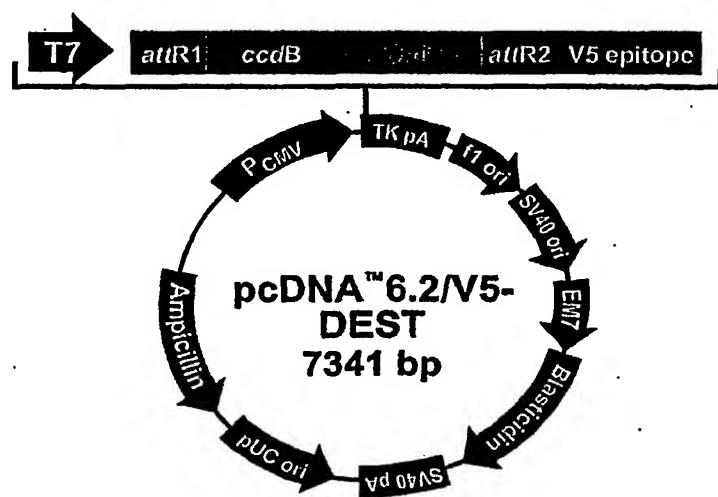


FIG. 58

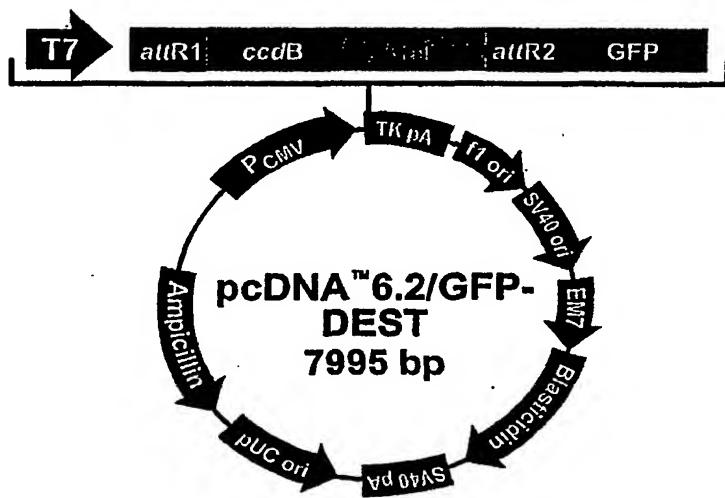


FIG. 59

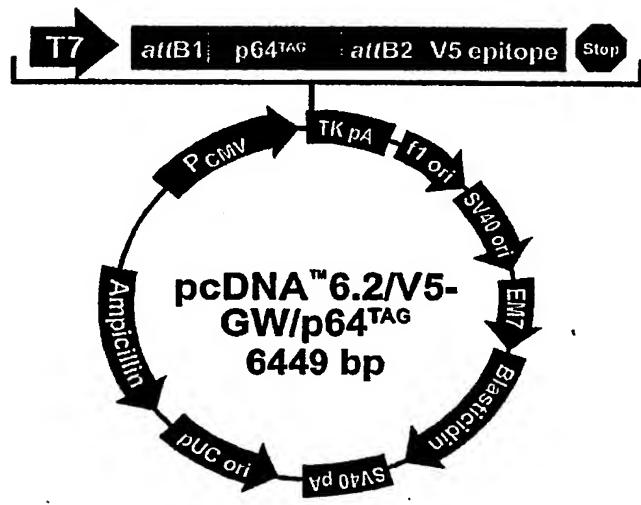


FIG. 60

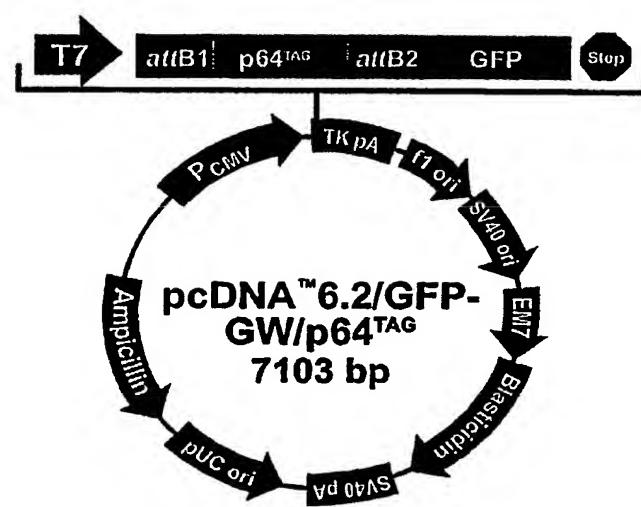


FIG. 61

A

CAAT TATA 3'end of CMV promoter Putative transcriptional start
 771 CAAATGGGCG GTAGGCCTGT ACGGTGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
 T7 promoter/priming site
 841 CTGCTTACTG GCTTATCGAA ATTAAATACGA CTCACATAG GGAGACCCAA GCTGGCTAGT TAAGCTATCA
 911 ACAAGTTT C TTG TAC AAA GTG GTT
 911 TGTCAAACA TGTTC TTT CAC CAA
 Pro Ala Phe Leu Tyr Lys Val Val
 3177 GAT CTA GAG GGC CCG CGG TTC GAA GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC
 Asp Leu Glu Gly Pro Arg Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu
 V5 epitope V5 reverse priming site
 3234 GAT TCT ACG CGT ACC GGT TAG TAA TGA GTTTAAACGG GGGAGGCTAA CTGAAACAGC
 Asp Ser Thr Arg Thr Glv *** *** ***

B.

CAAT TATA 3'end of CMV promoter Putative transcriptional start
 771 CAAATGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
 841 CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGT TAAGCTATCA
 911 ACAAGTTT C TTG TAC AAA GTG GTT
 918 atB1 3161 atB2
 TGTCAAACA TGTTC Pro Ala Phe Leu Tyr Lys Val Val
 Green Fluorescent Protein (cycle-3 GFP)
 3177 GAT CTA GAG GGC CCC GCG GCT AGC AAA GGA GAA GAA CTT TTC ACT GGA GGT GTC CCA
 Asp Leu Glu Gly Pro Ala Ala Ser Lys Gly Glu Leu Phe Thr Gly Val Val Pro
 3234 ATT CTT GTT GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG
 Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu.
 GFP reverse priming site
 3291 GGT GAA GGT GAT GCT ACA TAC GGA AAG CTT ACC CTT AAA TTT ATT TGC ACT ACT GGA
 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly
 3348 AAA CTA CCT GTT ...
 Lys Leu Pro Val ...

FIG. 62

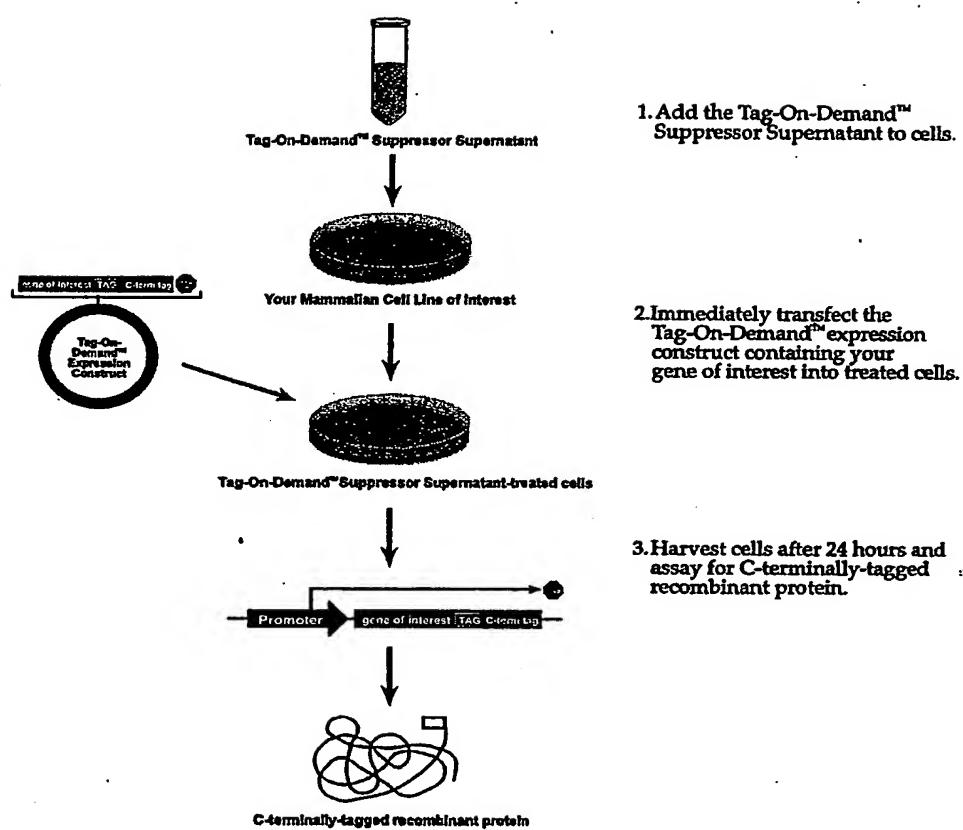


FIG. 63

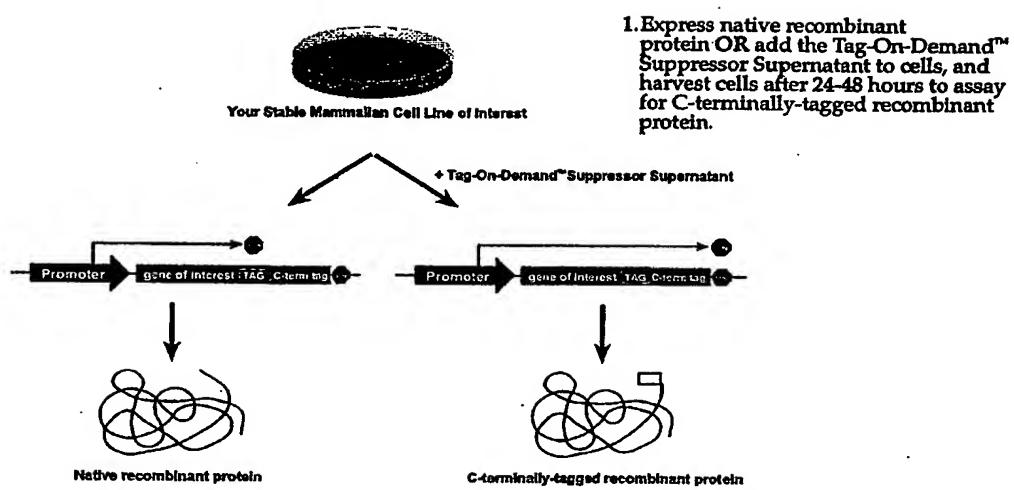
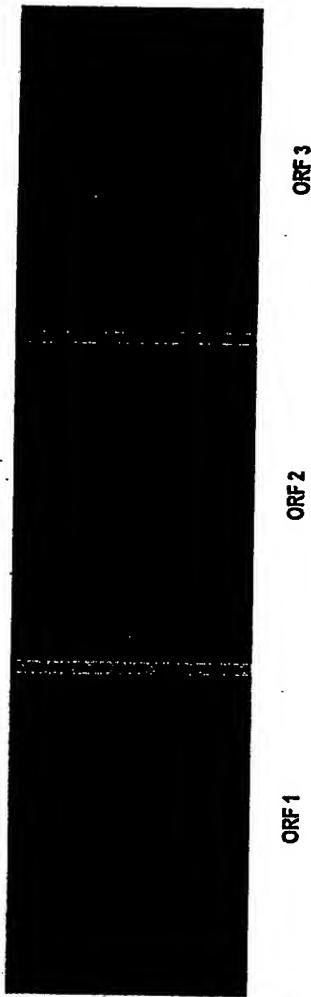


FIG. 64



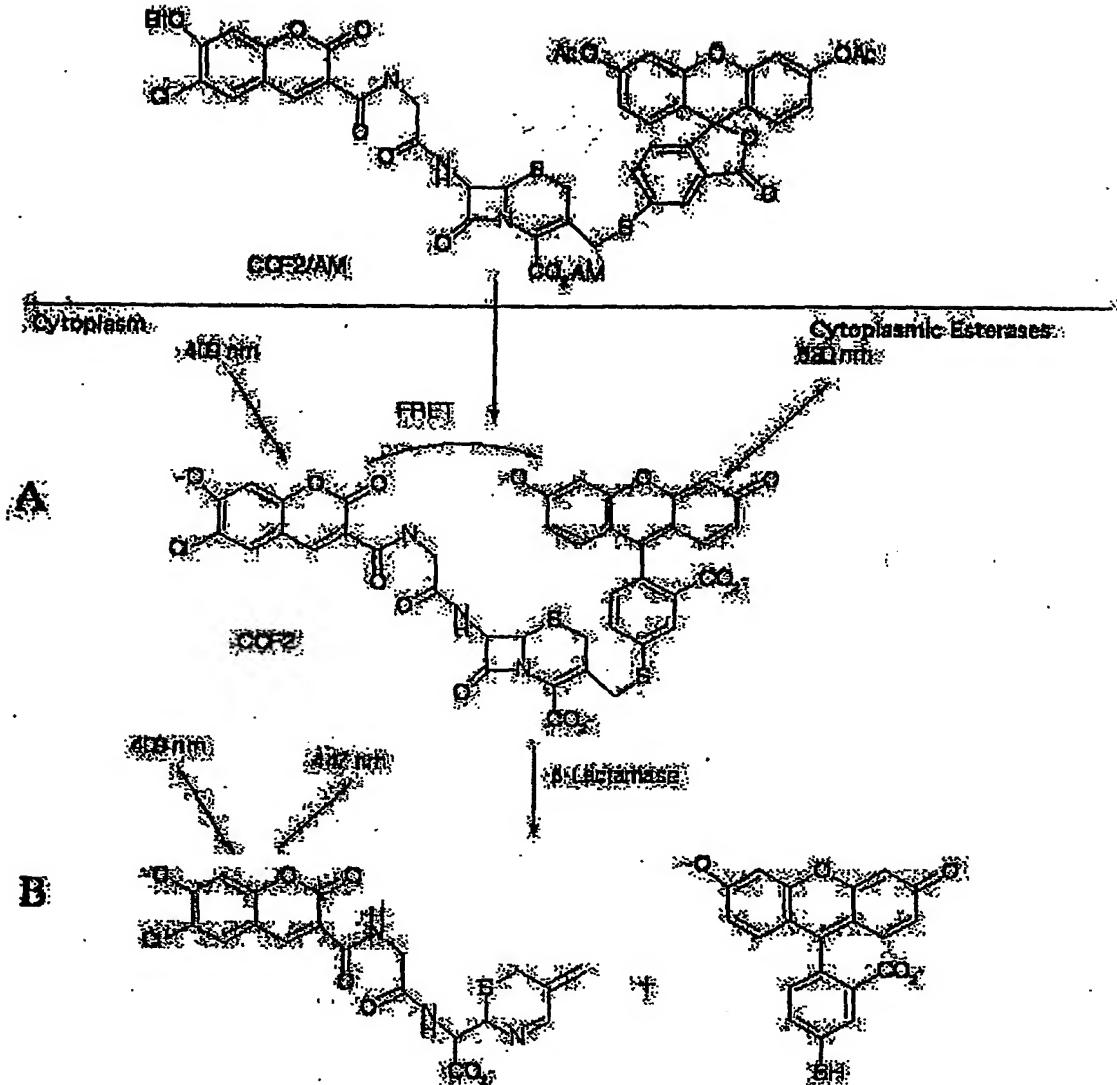
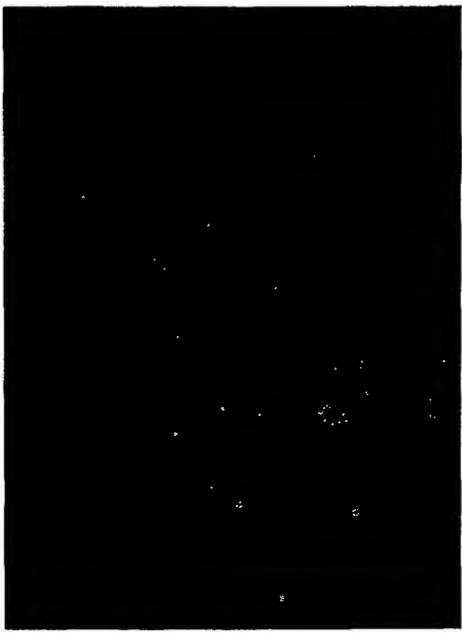
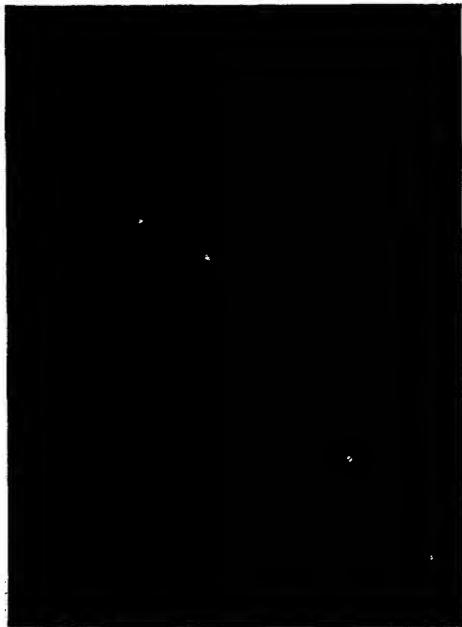


FIG. 65

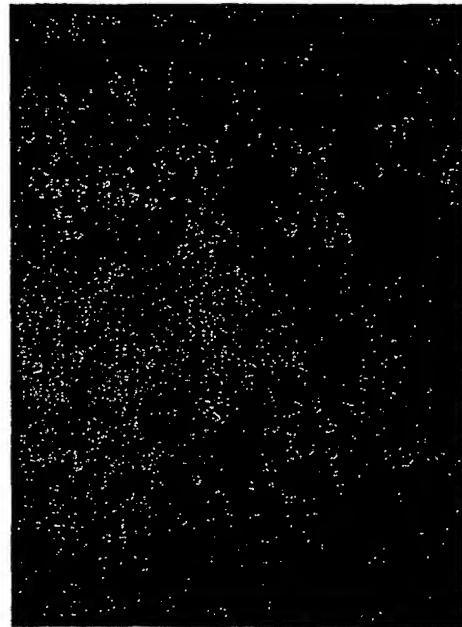
Fig. 66

Sequential method



Simultaneous method

Brightfield



Fluorescent

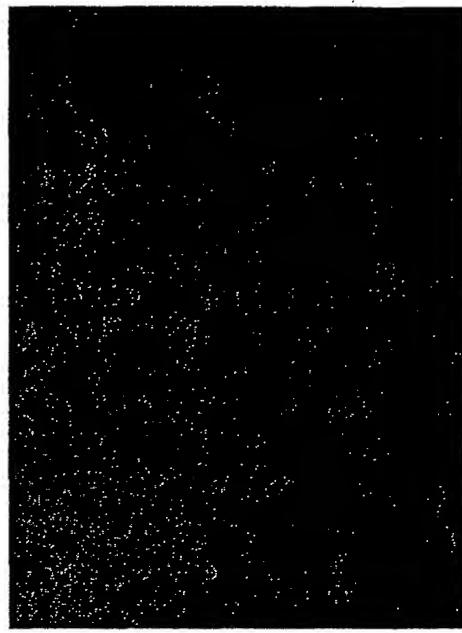
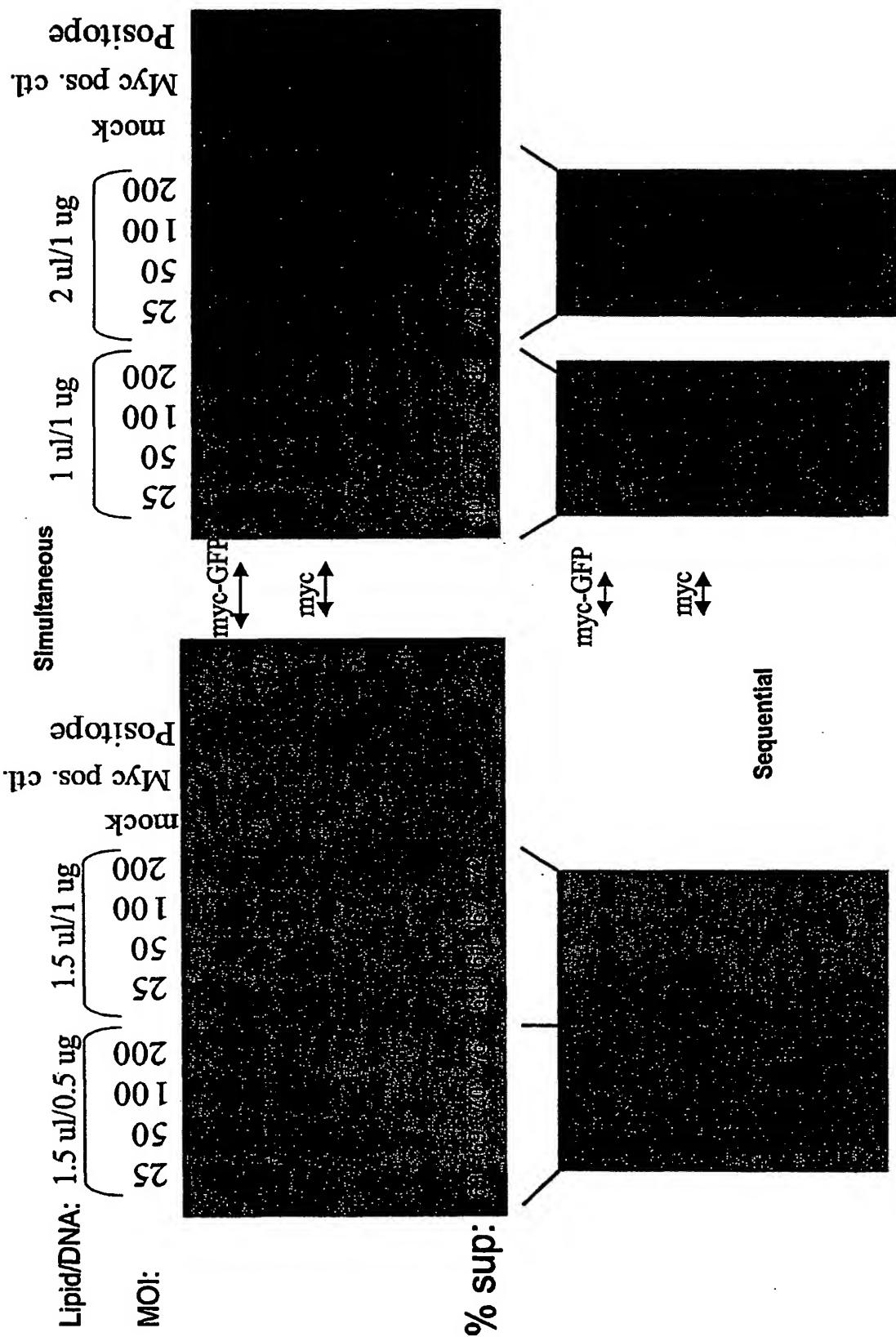


FIG. 67



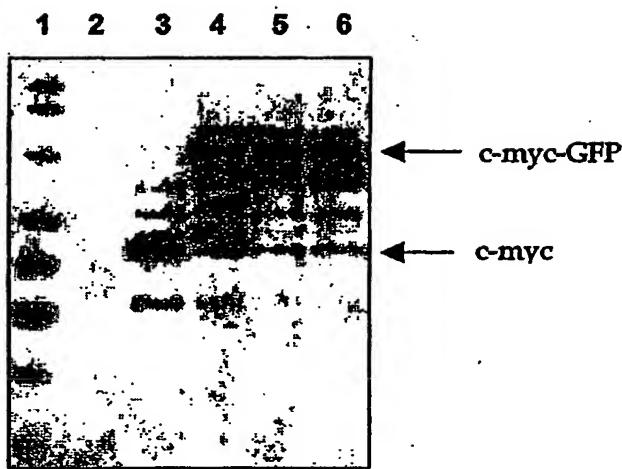


Fig. 6B

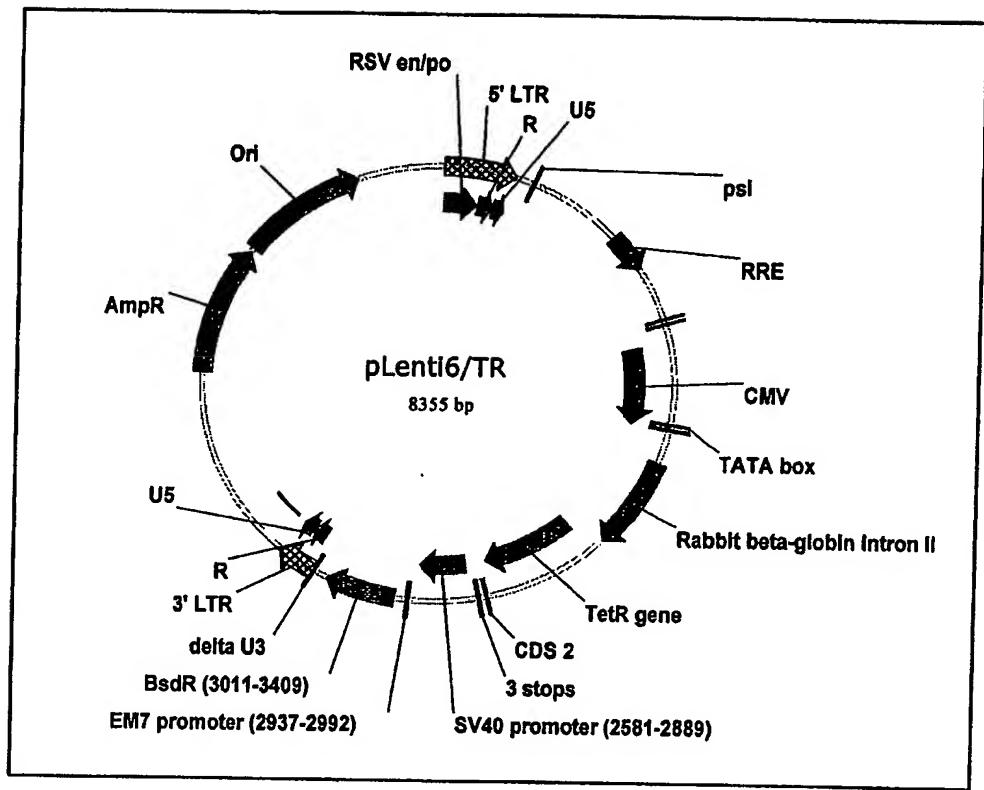


FIG. 69

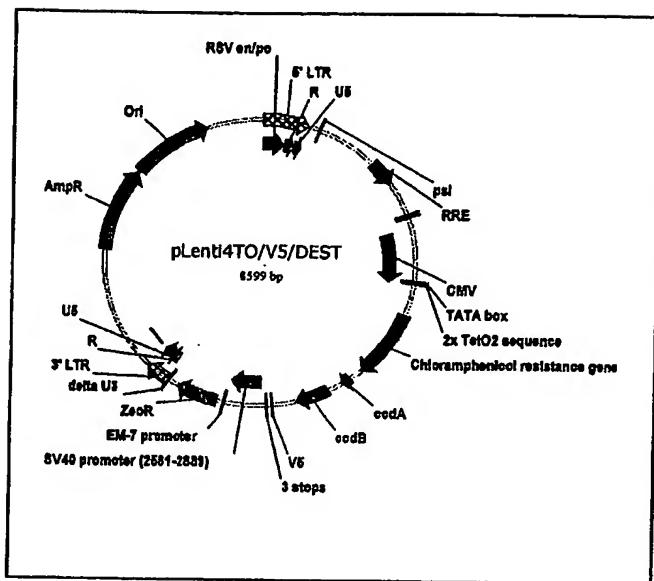


FIG. 70A

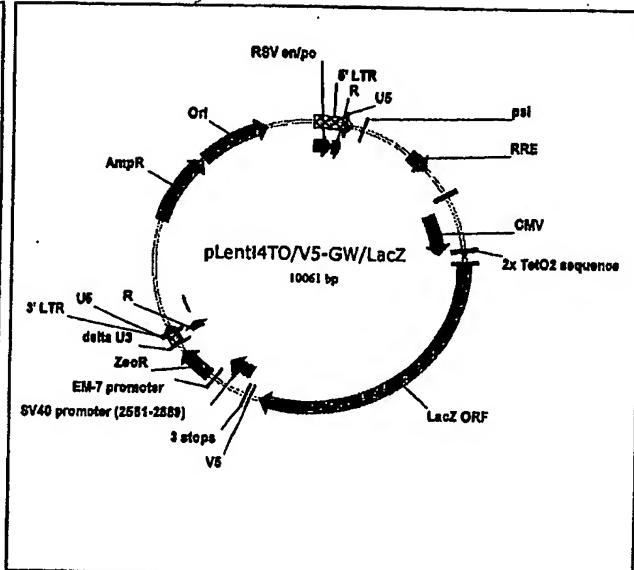


FIG. 70B

FIG. 71

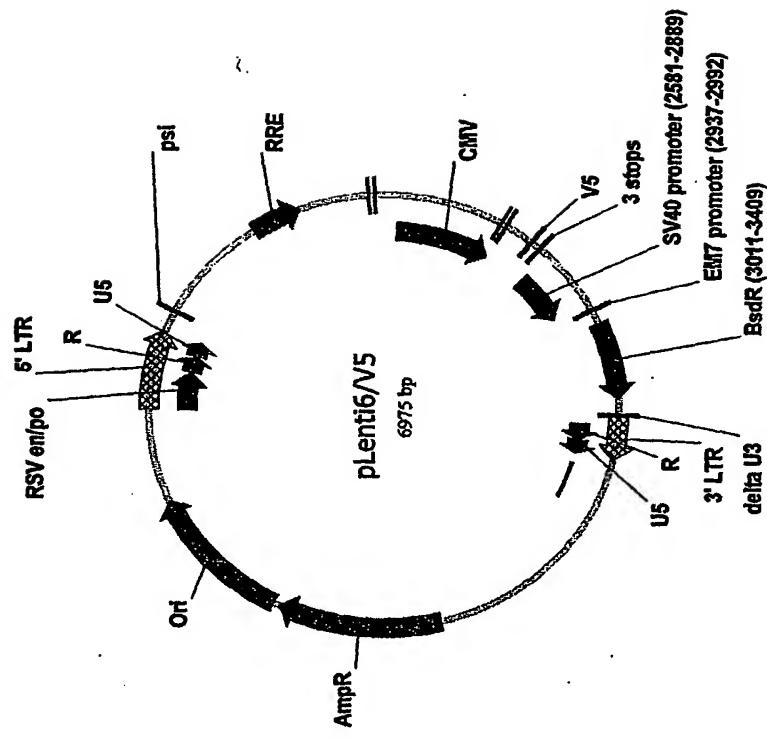


FIG. 72

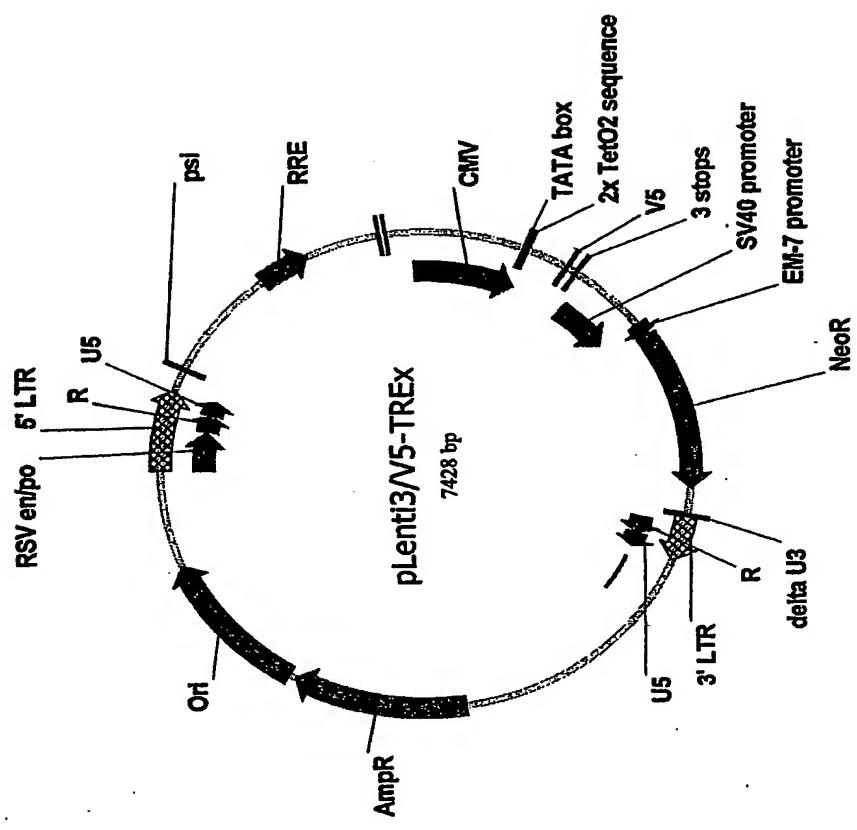


FIG. 73

